A COMPARATIVE STUDY OF MINERAL CONTENTS IN SELECTED MALAYSIAN BROWN RICE AND WHITE RICE

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FACULTY OF SCIENCE
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Abstract

In the present study, concentration of minerals Na, Mg, K, Ca, Mn, Fe, Cu and Zn in selected commercially available brown rice and white rice from Malaysia were determined using microwave assisted digestion with inductively coupled plasma mass spectrometry. The data obtained were evaluated using chemometric tools. Hierarchical cluster analysis and principal component analysis visually reveal the natural clustering of rice sample into two distinct clusters where brown rice are associated with higher mineral concentration. The statistics show that all the mineral concentrations found in brown rice are significantly higher than those found in white rice ($P<0.001$). As a whole, the mean concentration of minerals follow a trend of K>Mg>Zn>Mn>Ca>Fe>Na>Cu for brown rice samples and a trend of K>Mg>Zn>Ca>Mn>Fe>Na>Cu in white rice samples.

Abstrak

Dalam kajian ini, kepekatan mineral-mineral Na, Mg, K, Ca, Mn, Fe, Cu and Zn yang hadir dalam beras perang dan beras putih komersial terpilih daripada Malaysia ditentukan menerusi teknik penghadaman ketuhar bersuhu tinggi sebelum dikesan oleh *Inductive Coupled Plasma Mass Spectrometry* (ICP-MS). Analisis data berkenaan dinilai melalui kaedah *chemometric*. Analisis kelompok hierarki dan analisis komponen utama yang dijalankan menunjukkan kewujudan dua kelompok berbeza di mana beras perang didapati mengandungi kepekatan mineral-mineral yang lebih tinggi. Analisa statistik pula menunjukkan kepekatan semua mineral-mineral dalam beras perang mempunyai nilai yang lebih tinggi secara ketara ($P<0.001$) berbanding dengan beras putih. Secara keseluruhannya, kepekatan purata mineral-mineral mengikuti susunan K>Mg>Zn>Mn>Ca>Fe>Na>Cu untuk sampel beras perang dan susunan K>Mg>Zn>Ca>Mn>Fe>Na>Cu untuk sampel beras putih.

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List of Symbols & Abbreviations

Inductive Coupled Plasma Mass Spectrometry	ICP-MS
Hierarchical Cluster Analysis	HCA
Principle Component Analysis	PCA
Sodium	Na
Magnesium	Mg
Potassium	K
Calcium	Ca
Manganese	Mn
Iron	Fe
Copper	Cu
Zinc	Zn
Nitric Acid	HNO_3
Hydrochloric Acid	HCL
Ultra Pure Water	UPW
Microgram per gram	µg/g
Milligram per gram	mg/g
Mililitre	ml
Percentage	%
Degree Celcius	°C
Less than	$<$

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CHAPTER 1

INTRODUCTION

1.1 Food and Nutrition

Food is vital in our daily life, and without it one cannot survive. From the moment an individual is conceived until death, he or she requires supplies of energy (Fieldhouse, 1995). This energy is obtained via dietary supplements composed of elements arranged in a variety of ways to form various nutrient molecules (Housten, 2000). Nutrient molecules can be divided into two categories which are organic nutrients and inorganic nutrients. Examples of organic nutrients are carbohydrates, proteins, fats and vitamins. Meanwhile inorganic nutrients include dietary minerals, water and oxygen (Ensminger, 1994). Table 1.1 shows some examples of nutrients and its chemical formula.

Table 1.1: Nutrients and its chemical formula

Organic nutrients	Inorganic nutrients
Carbohydrates; $Cm(H_2O)n$ m, n: different numbers	Oxygen, O_2
Fats, Saturated; $CnH_{(2n+1)}CO_2H$ Unsaturated; $C_nH_{(2n-1)}CO_2H$	Water, H_2O
Protein; $NH_2C(R)HCOOH$ (amino acid)	Dietary minerals, Ca, P, K, S, Na, Mg, Fe, Cu, Zn, Mn, Se, Co, Ni, Mo
Vitamins; Vitamin A: $C_{20}H_{30}O$ Vitamin B: $C_6H_8O_6$,Vitamin D2: $C_{27}H_{44}O$	

The study of nutrients and their relationship with food and living things is called nutrition (Tull, 1996). Nutrition can also be defined as the interaction that occurs between food and the organism that consumes it (Housten, 2000). The source of nutrition can be obtained from a single food or a combination of different foods. Hence the importance of nutrition is unprecedented and a human lacking in essential nutrients are said to be malnourished (Grosvenor and Smolin, 2002). A healthy diet can be achieved using food pyramid as the guide. As shown in Figure 1.1, food such as rice, bread, cereal and pasta occupying the lowest level are to be consumed more than fruits and vegetables followed by the protein source at 2-3 servings and finally fats, oils and sweets occupying the highest level are to be consumed the least (USDA, 1992).

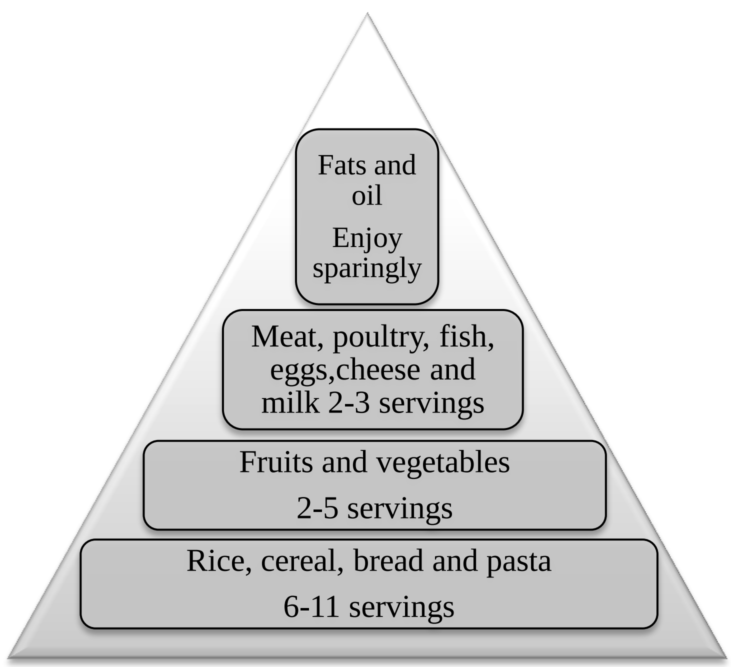

Figure 1.1: Food guide pyramid
Source: (USDA, 1992)

1.2 Dietary Minerals

Dietary minerals as mentioned above are nutrients required by the human body. They can be regarded as inorganic elements that function in the body as structural components and regulate body processes (Grosvenor and Smolin, 2002). These elements are essential for life growth and development as they mostly bound to proteins, forming metallo proteins, especially enzymes (Housten, 2000). Sometimes dietary minerals are also known as dietary elements or mineral nutrients. Most of the dietary elements come from the intake of grains like rice, wheat and maize (Gai *et al*., 2004).

Dietary minerals accomplish decisive functions to maintain human health, and their deficiency can lead to undesirable pathological conditions (Cesar, 2005). In average, humans need about 15 types of minerals from their daily diet, which can be divided into two groups known as macrominerals and microminerals based on the daily needs (Housten, 2000; Judith, 2008; Mateljan, 2009). Macrominerals are the major dietary elements that needed by human body with intake greater than 100 mg per day so as to sustain their body functions. They include Ca, P, K, S, Na, CI and Mg (Mateljan, 2009). On the other hand, microminerals or trace/minor dietary elements are required in an amount of 0.01 % or less of the body weight. These include Fe, Co, Cu, Zn, Mo, Mn, Se, I and Ni (Mateljan, 2009). In this regard, it is advisable to control the intake according to the recommendation for adequate and safe levels (Housten, 2000). This can be achieved through regular intake of a balanced diet which is made up from variety of foods (Jerome, 2007).

Basically, for all age of human, an adequate intake of minerals is not only essential for a high nutritional quality of the diet, but also contributes to the prevention of chronic nutrition related diseases (Jerome, 2007). Entering the second decade of the 21st century, experts have agreed that the world faces three major challenges. The first is development of decreased malnutrition and infectious disease. The second is the alarming increase in the incidence of chronic diseases like heart disease, Type II diabetes, obesity, and cancers in developing nations. The third is a consequence of globalisation whereby traditional diets are being replaced with nutritionally compromised fast-foods (Dipti *et al*., 2012). Mineral deficiency is a feature of each of these challenges, and in a sad twist of irony, developing countries, where rice is the staple, are the hardest hit by all three of these global challenges.

1.3 The Background of Rice

Rice *(Oryza sativa L.)* is the staple food for 40% of the population in the world (Parenga *et al*., 2010). It is one of the most important cereals in human nutrition that consumed by about 75% of the global population and provides 60% of the food intake in Southeast Asia (Anjum *et al*., 2007). In fact, the average rice consumption per capita in Malaysia is about 203g daily (FAO, 2009). It is not just a very important source of energy, but also contributes minerals, vitamins and amino acids needed by the body. Since rice is a food of great importance in the human diet, the nutritional assessment of rice provides data relevant to nutritionists. The dietary minerals in rice include Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn and Se (Silva, 2013).

It is generally agreed that human population around the world consume higher amount of white rice compared to brown rice. Basically, brown rice is an unpolished whole grain rice with only the hull removed and retains the nutrient-dense bran layers that give it a brown color, chewy texture and nutty flavor (Hansen *et al*., 2012). Meanwhile in white rice, its husk, bran, and germ are all removed (Silva *et al*., 2013). For that reasons, brown rice seems to contain higher nutrients than milled or polished white rice (FAO, 2001). Likewise, there is definite difference in term of the minerals (Janet *et al*., 2002; Anjum, 2007). According to Senadhira *et al*., (1998), the variation in the mineral content may also depend upon the processing of rice, the quality of soil and fertilizer used in rice cultivation. In this regards, will the contributions from such factors mask the mineral variations that associated with brown rice and white rice?

In this study, the content of Na, Mg, K, Ca, Mn, Fe, Cu and Zn will be determined in selected Malaysian brown rice and white rice by microwave assisted digestion-inductively coupled plasma-mass spectroscopy (MAD-ICP-MS). The results obtained can be further evaluated with chemometric tools so as to explore the pattern associated with the rice samples. In addition, the experimental values obtained can be compared with Recommended Daily Intake (USDA, 2010) values. We hope the knowledge gained in this study will create awareness among consumers about the amount of minerals they consume and if it adheres to the values.

1.4 Introduction to Chemometrics

Modern automatic analysis methods provide opportunities to collect large amounts of data very easily particularly the chromatographic and spectroscopic techniques can provide analytical data on many components on a single specimen. In other words, masses of data can be collected in every field, but the ability to use these data efficiently to make an intelligent decision is a challenge (Miller, JN. and Miller JC. 2005). For example, in this study the ICP-MS allows simultaneous measurement of various mineral concentrations for each sample, which yields multivariate data which make it difficult to see their patterns and relationships with naked eyes or sorted out by traditional univariate approaches. In this regard, chemometric which encompasses the application of mathematical and statistical methods, provides an alternative to extract useful information from such data (Volmer, 2001). In this context, principal component analysis (PCA) and hierarchical cluster analysis (HCA) are common options to explore the underlying structure in selected Malaysian rice samples based on their mineral contents.

1.4.1 Hierarchical Cluster Analysis (HCA)

HCA is a major statistical technique for classifying a group of samples into manageable sub-groups based on the dissimilarity (Beebe, 1998). In this approach, there is no prior knowledge about which samples belong to which clusters (Kaufman, 2005). The groupings or clusterings can be developed via predefined algorithm example the dissimilarities or distances between samples. For example, single linkage clustering can start with each sample as a separate cluster, there are as many clusters as samples, and then combines the clusters sequentially, reducing the number of clusters at each step until only one cluster is left (Everitt, 2001). A hierarchical tree diagram, called a dendrogram, can be produced to show the linkage points clusters can be linked at increasing levels of dissimilarity (Beebe, 1998). Such dendrogram facilitates the exploration of natural grouping among the samples residing in them. In this context, the HCA can be used to transform the multivariate ICP MS data into a manageable dendrogram that eases interpretation. The points that lie close to one another denotes samples that share similar mineral values (Beebe, 1998).

1.4.2 Principle Component Analysis (PCA)

PCA is primarily a mathematical method for data reduction and it does not assume that the data have any particular distribution (Miller, JN. and Miller JC. 2005). It transforms the original data matrix into a product of two matrices, the score matrix contains information about samples while the loading matrix about variables as shown in Figure 1.2. These matrices ease the perception of latent structures as the dimensionality of the data has been reduced. In other words, PCA is a useful technique for transforming a large number of variables in a data set into a smaller and more coherent set of uncorrelated (orthogonal) factors, the principal components. The principle components account for much of the variance among the set of original variables, where the first component accounts for the largest possible amount of variation in the original variables (Jolliffe, 2002). The second component is completely uncorrelated with the first component, and accounts for the maximum variation that is not accounted for the first. The third accounts for the maximum that the first and the second not accounted for and so on (Beebe, 1998). Each PC is a linear weighted combination of the initial variables, where the weights are given by the eigenvectors. In this study, the score plot gives an overview on how the rice samples are related to each other in term of their mineral content.

Meanwhile the loading plot reveals the relationships between the studied minerals in the space of selected principles components. By mapping these two plots, allows visual exploration of the relationship between the rice samples and the minerals.

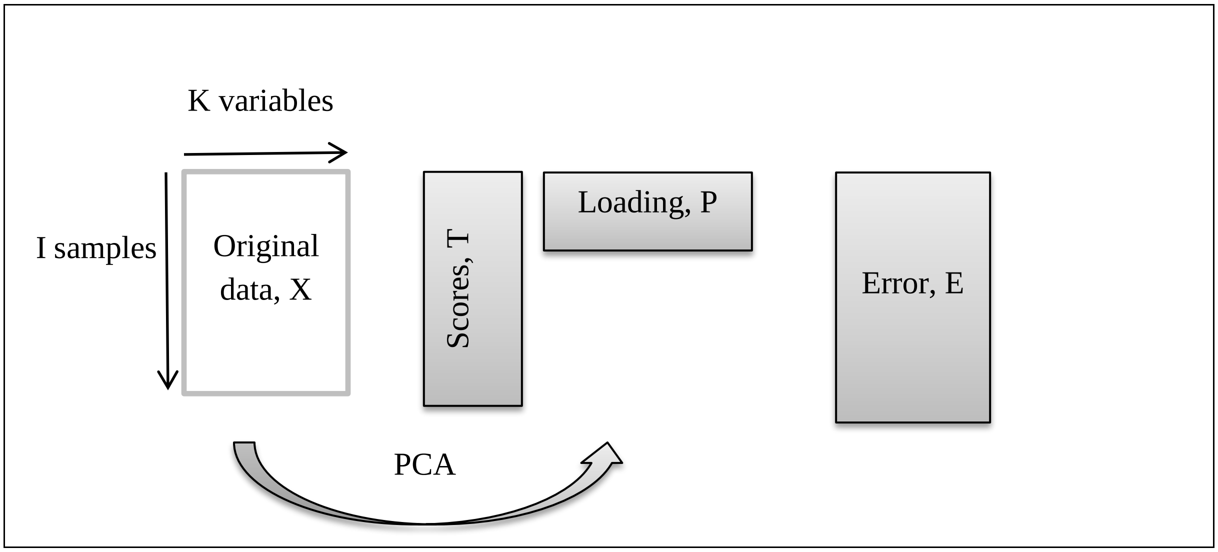

Figure 1.2: Conceptual illustration of PCA

1.5 Objectives of study

This study aims to explore if there is any notable trend present in mineral content of Malaysian brown rice and white rice and their contribution to the Malaysian public's nutrition intake. Within the study, we may also identify sub-objectives that will complement each other in order to achieve the main objective.

1. To determine and to compare the concentration of total Na, Mg, K, Ca, Mn, Fe, Cu, and Zn in selected Malaysian brown rice and white rice samples.

2. To explore the link between the studied minerals and the rice samples.

3. To assess their contributions to Recommended Daily Intakes (USDA, 2010).

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Rice

As mentioned in previous section, Rice *(Oryza sativa L.)* is a very important source of energy other than to contribute vitamins, minerals and amino acids needed by the body. In fact, it is estimated to provide 20% of the world's dietary energy supply compared to wheat supplies 19% and maize 5% (Suzanne, 2003). Based on literature survey, rice is consumed by about 75% of the global population and regarded as the staple food for 40% of the population in the world (Parenga *et al*., 2010) and contributes 60% of the food intake in the Southeast Asia (Anjum *et al*., 2007). According to FAO (2004) report, rice is regarded as a basic daily food for 17 countries in Asia and the Pacific, nine countries in North and South America and eight countries in Africa (FAO, 2004). In fact, its average consumption in Asia is about 81 kg per year (Kunlun *et al*., 2009). For that reason, the major rice growing areas are found in China, India, Indonesia, Bangladesh, Thailand, Burma, Vietnam, Japan and Philippines (Amissah *et al*., 2002).

There are two types of rice that commonly found in the market namely “brown rice” and “polished rice” or “white rice”. According to Anjum (2007), there is definite difference in term of vitamins, minerals, fiber and fat contents exists between them (Anjum, 2007). Basically world population consume higher amount of latter compared to the former.

2.2 The Rice Production

Rice is a staple crop and forms the foundation of the diet for many of the world's population, especially those living in Southern and Eastern Asia. It is a very important crop in Asia as archaeological sources has shown that rice has been planted before the 4000 AD. Sources said that 90% of the production of rice produced in the world was grown in Asia (Suzanne, 2003). According to the Association of Japanese Agricultural Scientific Societies (1975), every continent on the planet produces rice continent except Antartica (Amissah *et al.*, 2002).

Rice is grown in tropical and subtropical areas where the relative humidity of this area and the rainfall is high. Basically rice can be divided into three groups, that is japonica, indica, and javanica (Sergio, 2010). There are a huge number of rice varieties such as long grain, basmati and Arborio but only a few are grown widely. The countries that produce rice are China, India, Indonesia, Bangladesh, Thailand, Burma, Vietnam, Japan, and Philippines (Suzanne, 2003).

Basically, the rice production in Malaysia has been increasing from year to year. In Malaysia the area of rice production covers all states and is predominantly grown in Kedah which known as the “rice bowl” of Malaysia. Table 2.1 shows the rice production in metric tonnes from 2007 to 2011 for all states.

Table 2.1: Rice production in Malaysia

State	Rice production in Metric Tonnes (Mt)				
	2007	2008	2009	2010	2011
Johor	9,221	8,128	9,659	11,224	11,477
Kedah	911,295	867,335	923,666	835,630	878,430
Kelantan	249,440	232,309	265,289	271,300	272,805
Melaka	7,225	4,158	5,550	5,071	7,505
N. Sembilan	5,091	5,437	7,289	8,830	6,447
Pahang	22,673	21,384	30,084	25,312	27,110
Perak	259,081	280,237	311,150	294,705	323,445
Perlis	198,025	233,144	235,682	222,884	232,674
P.Pinang	120,286	120,075	133,048	142,434	144,613
Selangor	186,951	177,444	202,633	210,292	221,295
Terengganu	62,253	63,490	69,590	74,962	77,796
Sabah	134,384	133,138	131,710	147,531	129,722
Sarawak	209,679	206,753	185,693	214,655	242,669
Malaysia	2,375,604	2,353,032	2,511,043	2,464,830	2,575,988

Source: (Department of Agriculture, Malaysia, 2012)

2.3 Rice Botany and Classification

Rice has 8000 different varieties botanically and more than 4000 varieties have been identified. *Oryza sativa L.* is one member of the family Poaceae. There are 20 species found within the genus *Oryza* but only one species is produced widely that is Oryza sativa L. (Mahmoud *et al*., 2008). The height of the plants is as high as 40 to 700 cm. In modern times now, the height of rice plants can be achieved to 100 cm. *Oryza Sativa* usually divided into two subspecies that is japonica and indica. According to (Mahmoud *et al*., 2008) rice classification is based on their morphological differences and responses to temperature and length of day. In the Asia, the subspecies crosses is very common where if frequently produces plants with a high degree of sterility (Samuel, 1991).

Freshly harvested rice is called paddy grain or rough rice. The pearly white starch grain used for cooking is the centre of the rice seed and is covered and protected by the hull. Inside the hull, the familiar white grain is covered by a layer called bran. The embryo, a small structure at the base of the grain, is also contained within the bran layer. Together, the grain, embryo and bran are called brown rice. Figure 2.1 shows the structure of rice grain.

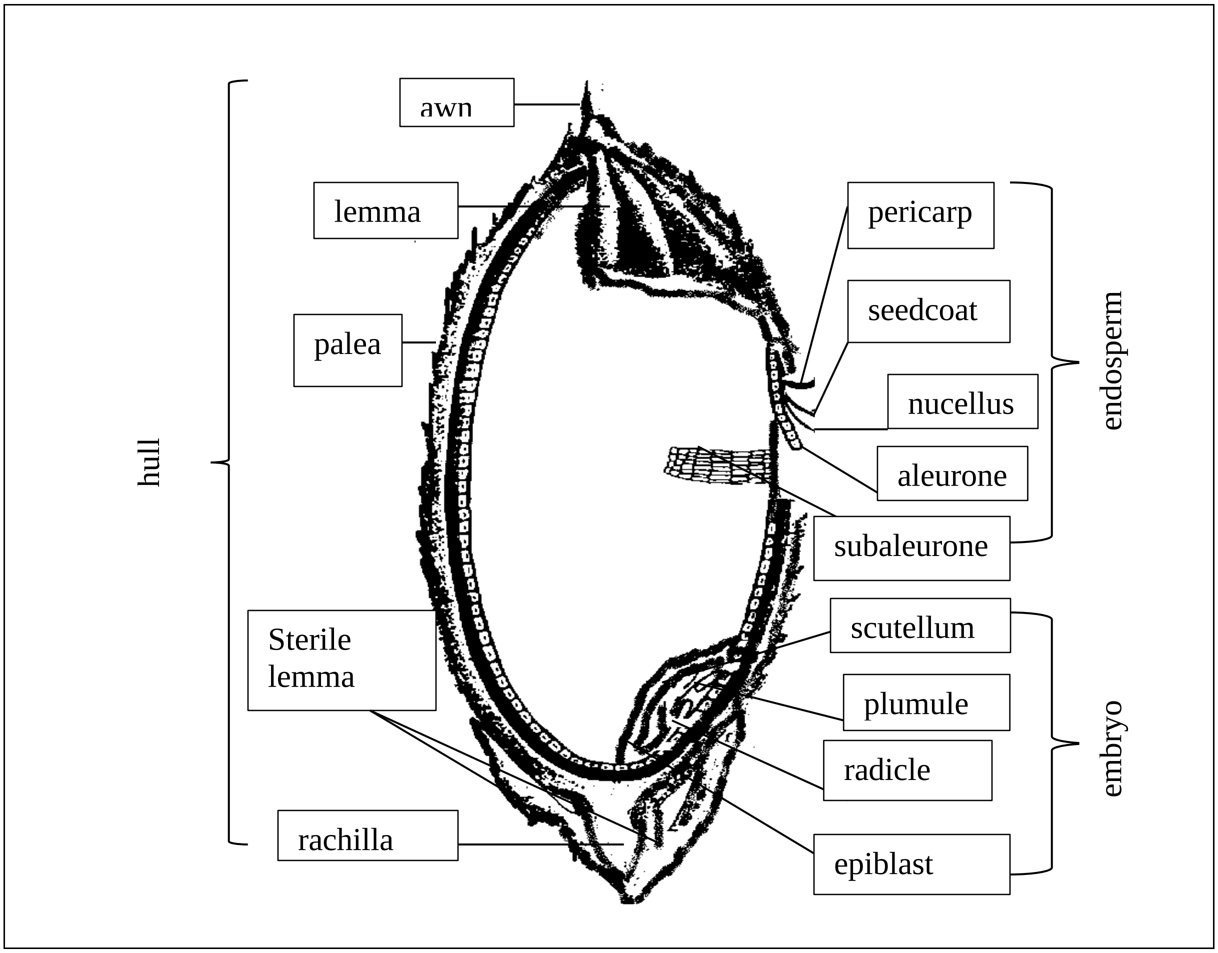

Figure 2.1: Structure of rice grain
Source: Food and Agriculture Organization, (1993)

2.3.1 Brown Rice

Brown rice is an unpolished whole grain rice with only the hull removed and retains the bran layers that gives it a brown color, chewy texture and nutty flavor. It consists of the outer layer pericarp, seeds, nucellus, embryo and endosperm (Juliano, 1993) in which about 2% pericarp, seed coat and aleurone about 5%, germ 2 to 3% and endosperm about 89 to 94% (Gene, 1991). It is therefore generally rich in nutrient, especially B-complex group compared to polished rice (Janet *et al*., 2002). In fact, B-vitamins and α-tocopherol (vitamin E) are concentrated in the bran layers with the embryo accounting for more than 95% of total tocopherols.

For instance, it has more than three times the fiber of white rice which is beneficial to the human health. A cup of brown rice provides 14.0% of the daily value for fiber (Liu, 2002). So by consuming this brown rice in for a continuous period, it had been shown to reduce high cholesterol to prevent atherosclerosis (McKeown *et al*., 2002). Brown rice is chosen as excellent grain choice for diabetic people as the fiber helps in maintains the blood sugar level under control (Rukmini and Raghuram, 1991). In addition, the fiber in brown rice help to protect against colon cancer since fiber binds to cancer-causing chemicals, keeping them away from the cells lining the colon, henceforth it can help normalize bowel function, reducing constipation (World's Healthiest Foods, 2009). Brown rice has also been identified as a rich source of Mg, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in utilization of glucose and secretion of insulin (World's Healthiest Foods, 2009).

A cup of brown rice will provide up to 88.0% of the daily value for manganese. (McKeown *et al*., 2002). This trace mineral helps produce energy from protein and carbohydrates and is involved in the synthesis of fatty acids, which are important for a healthy nervous system, and in the production of cholesterol, which is used by the body to produce sex hormones (Liu, 2002). Mn is also a critical component of a very important antioxidant enzyme called superoxide dismutase. Superoxide dismutase (SOD) is found inside the body's mitochondria (the oxygen-based energy factories inside most of our cells) where it provides protection against damage from the free radicals produced during energy production (World's Healthiest Foods, 2009). In addition, this brown rice is also a major source of thiamin and niacin (Parenga *et al*., 2010). According to the American Journal of Clinical Nutrition, in order to maintain a healthy body weight people are advised to consume brown rice rather than the polished rice.

2.3.2 White Rice

White rice is the name given to milled rice or polished rice that has had its husk, bran, and germ removed (Janet *et al*., 2002). This alters the flavour, texture and appearance of the rice and helps prevent spoilage and extend its storage life. Also during whitening process, the outer bran is removed leaving the core component of mostly carbohydrate remaining. These processes change the composition and certain nutrients significantly which may create nutritional hazard. Among the nutrients often found deficient in diets of white rice-eating people are vitamin B1 and lysine (Parenga *et al*., 2010).

2.3.2.1 Potential Losses of Minerals during Milling

Polishing is an important operation leading to the production of white rice. The degree of polishing has a significant effect on the quality and nutritional aspects of white rice, affecting properties such as content of essential minerals (Liang *et al*., 2008). The outer grain layers are much denser in minerals than the inner parts, causing a substantial decline in the concentration of mineral elements in the polished grain.

Nevertheless, polishing is a preferred step in postharvest production of rice because most consumers prefer the taste and texture of the white rice and the high oil content of the bran increases risks of rancidity, thereby reducing the shelf life. In addition, polishing is carried out because it decreases the cooking time, which is an important property for poor rice consumers (Fitzgerald *et al*., 2009).

The intensity of polishing depends on the type of mill and the duration of the treatment (Graves *et al*., 2009; Roy *et al*., 2011). The relationship between polishing time and loss of material is nonlinear due to increasing adhesion of the bran layers from outer to inner layers, while the different endosperm fractions seem to have comparable hardness (Lamberts *et al*., 2007). Furthermore, differences in shape and size of rice grains also contribute to the quantity of material lost during polishing (Liang *et al*., 2008). The loss of mineral elements associated with polishing of rice is not directly related to the loss of grain mass because elements have a heterogeneous distribution within the grain (Lombi *et al*., 2009).

The effects of polishing on losses of macrominerals and microminerals have been studied in rice grain subjected to different polishing rates (Bryant *et al*., 2005; Itani *et al*., 2002). These studies generally show decreased concentrations of macronutrients moving from the outer edge to the centre of the grain, as is also the case for micronutrients, although with some marked differences among elements (Lamberts *et al*., 2007; Ogiyama *et al*., 2008). Polishing characteristics are also important in order to reveal the distribution of mineral elements in relation to other grain factors affecting their bio-availability such as phytic acid (Liang *et al*., 2008). Table 2.2 shows the general mineral composition of brown rice and white rice obtained from USDA Nutrient Database, (2004). In this regards, some minerals (particularly K, Mg, Mn, and Zn) in brown rice with concentration over twice of polished rice could well be associated with higher risks of chronic diseases (Jiang *et al*., 2007; Zeng *et al*., 2008).

Table 2.2: Mineral composition of brown rice and white rice

Minerals	(µg/g)	
	Brown rice	White rice
Na	70	50
K	2230	1150
Mg	1430	250
Ca	230	280
Mn	37.4	10.9
Fe	14.7	8
Zn	20.2	10.9
Cu	2.8	2.2

Source: USDA Nutrient Database, (2004).

2.4 Recommended Daily Intake (RDI)

The Recommended Daily Intake (RDI) is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97–98% of healthy individuals in every demographic in the United States where it was developed, but has since been used in other place (Heinemann *et al.*, 2005). The RDI is used to determine the Daily Value (DV) of foods. As such RDI values are often used to assess contribution of minerals of a particular food source. According to Welch (2002), many of the important food systems used in developing countries fail to produce enough nutrients to sustain human requirements for healthy, active and productive lives. By combining the nutritional care of the population with the improvement of agricultural yield, sustainable solutions for the malnutrition problems can be created. Table 2.3 below shows the daily intake estimates for minerals. Estimates are for adults aged 19 to 50 with (M) representing male and (F) representing female.

Table 2.3: Daily intake estimates for minerals

Mineral	RDI, mg/day (M)	RDI, mg/day (F)
Ca	1000	1300
Cr	35	25
Cu	0.9	0.9
Fe	8	18
K	4700	4700
Mg	420	320
Mn	2.3	1.8
Mo	0.045	0.045
Na	2300	2300
P	700	700
S	850	850
Se	0.055	0.055
Zn	11	8

Source: (USDA, 2010)

CHAPTER 3

METHODOLOGY

3.1 Background

All the laboratory analysis for this study was conducted at level 5, Research Laboratory, Department of Chemistry (New Building), Faculty of Science, University Malaya. Figure 3.1 shows the workflow of the study conducted.

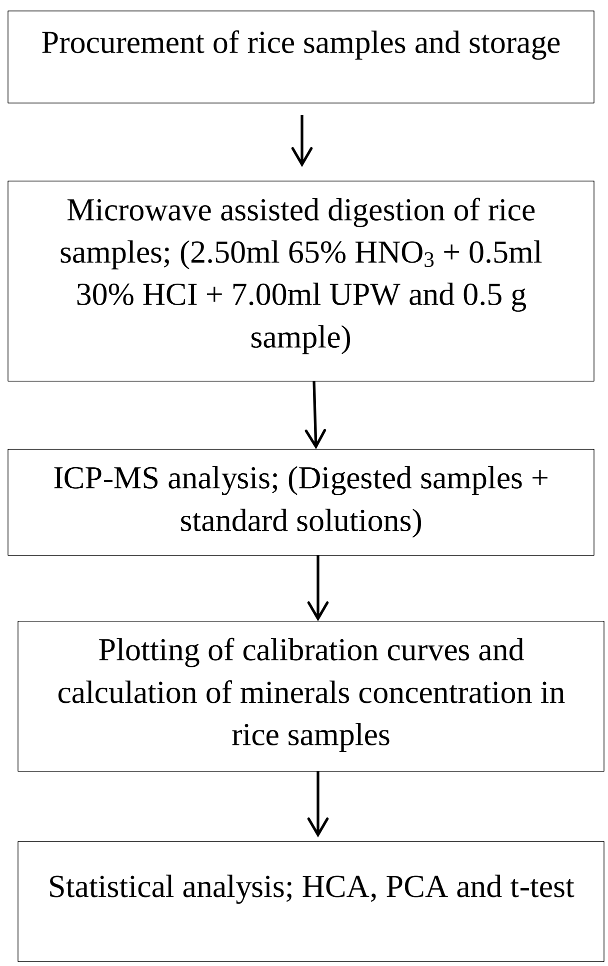

Figure 3.1: Workflow diagramme

3.1.1 Sampling and Preparation

Commercially available rice samples were purchased from several supermarkets in the area of Petaling Jaya, Malaysia. In total, 3 different brands of local brown rice (BRA, BRB, BRC) and 5 different brands of local white rice (WRA, WRB, WRC, WRD, WRE) were selected. A portion of each sample was transferred to a plastic container, labelled and was kept under room conditions before microwave digestion. The rice samples were directly used in analysis without prior treatment to avoid alteration of their mineral concentrations.

3.1.2 Apparatus

I. PTFE vessel
II. Polyethylene (PE) tubes
III. 50 mL polypropylene (PP) volumetric flask
IV. Multi pipette (20 μL to 10 mL)
V. Plastic bottle with cap (Sample Container)
VI. Plastic Funnel
VII. 20 and 50 mL plastic beaker
VIII. 2 L volumetric flask
IX. 5 mL and 10 mL measuring pipette

3.1.3 Reagents

All reagents were of at least analytical reagent grade unless otherwise stated. Nitric acid (65% HNO_3, Suprapur Merck, Germany), hydrochloric acid (30% HCl, Suprapur Merck, Germany), ultrapure water (UPW) (ELGA, UK) with resistivity of ≥18 MΩ cm was used throughout the experiments including for all dilutions and for rinsing.

All plastic containers and glassware were cleaned by soaking overnight in 10% (v/v) HNO_3 and were rinsed with ultrapure water prior to use. All standards, reagent solutions were kept in plastic containers and stored in the refrigerator before the measurements. A multi-element standard solution IV for ICP-MS (Agilent Technologies, USA) was used to prepare the series of calibration solutions for Zn, Mn, Cu, Na, Ca, Mg, K and Fe.

3.1.4 Preparation of Standard Solution

A series of multi-elemental calibration solutions of 0, 10, 30, 50, 75, and 100 μg/L of each Zn, Mn, Cu, Na, Ca, Mg, K and Fe were prepared by appropriate dilution with 5% nitric acid. These solutions were freshly prepared prior to the ICP-MS analyses.

3.1.5 Microwave Digestion

About 0.5 g of rice sample was weighed accurately and directly into each 55 mL self-regulating pressure control PFA vessel and digested with reagents consisting of 2.50 ml 65% HNO_3, 0.5 ml 30% HCL, and 7.00 ml UPW (Low *et al*., 2010). The digestion vessels were closed and heated in the CEM MarXpress Microwave Accelerated Reaction System

(CEM Corporation, NC, USA). After digestion, the obtained solutions were allowed to cool down to room temperature, and then were filtered through Whatman No. 1 (11 μm pores size) filter paper into a 50 mL PP volumetric flask. The volume was made up to the mark with UPW. The diluted sample solutions were stored in PE tubes below 8 °C before analysis by ICP-MS. The digestion was done simultaneously in replicates of 7 including one blank digestion for each rice sample. The microwave digestion setting is based on the parameters shown in Table 3.1.

Table 3.1: Microwave digestion setting (Low *et al*., 2010)

Power (W)	% max	Time (min) to raise temperature	Temperature (°C)	Running time (min)
1600	50	10.5	185	14.5

3.1.6 ICP-MS Analysis

ICP-MS is a widely used instrument adopted for the fast determination of multi-elements in samples. It can identify and quantify minerals with higher sensitivity due to relatively low detection limits. The concentrations of Zn, Mn, Cu, Na, Ca, Mg, K and Fe in all 64 samples studied including 8 blanks without samples were determined by an ICP-MS Agilent 7500A series (Agilent Technologies, USA). The relevant operating parameters and the setup information are shown in Table 3.2 and Table 3.3.

Table 3.2: ICP-MS operating conditions

ICP-MS operating conditions	Parameter
Plasma RF power	1,550 W
Reflected power	<15 W
Sampling depth	8.0 mm
Plasma gas flow	15 L/min
Carrier gas flow	1.2 L/min
Collision gas type	He
Collision gas flow	3.0-5.0 L/min
S/C temperature	2°C
Sampler and skimmer cones	Ni

Table 3.3: Setup information for all minerals and masses

Minerals	Mass	Mode	Integration Time (sec)
Na	23	He	0.15
Mg	24	He	0.15
K	39	He	0.15
Ca	43	He	0.30
Mn	55	He	0.30
Fe	56	He	0.30
Cu	63	He	0.30
Zn	66	He	0.30

Source: (Heinemann *et al*., 2004)

The analyzing steps by this instrument were started with the standard solution measurement and calibration curve plotting then followed by the measurement step to determine the elemental concentrations of the samples. Taking the blank into account, the elemental concentrations are calculated following (EPA 6020 method, 1994) as shown in Equation 1.

$$\textbf{Concentration } (\mu g/g) = \frac{C \times V}{W}$$

Equation 1
Source: (EPA 6020 method, 1994)

Where,

C = The concentration of the diluted digestion solution by ICP-MS (µg/L)

W = The amount of rice sample used in the microwave digestion (g)

V = The volume of the diluted digestion solution (L)

3.1.7 Statistical analysis

All the analyses were carried out using the JMP Pro 10.2.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Hierarchical Cluster Analysis

The data obtained by analysis of a total of 21 sub-samples of brown rice and a total of 35 sub-samples of white rice were evaluated by HCA. The results are shown as the dendrogram in Figure 4.1. Formation of two distinct groups can be observed. The green coloured cluster labeled B indicates brown rice and red coloured clusters along with label W indicates white rice. The rice samples that lies close to one another expected to share similar values in their minerals contents (Beebe, 1998). The pattern observed in the dendrogram suggested that the concentrations of Na, Mg, K, Ca, Mn, Fe, Cu, and Zn are good candidates to distinguish between brown rice and white rice.

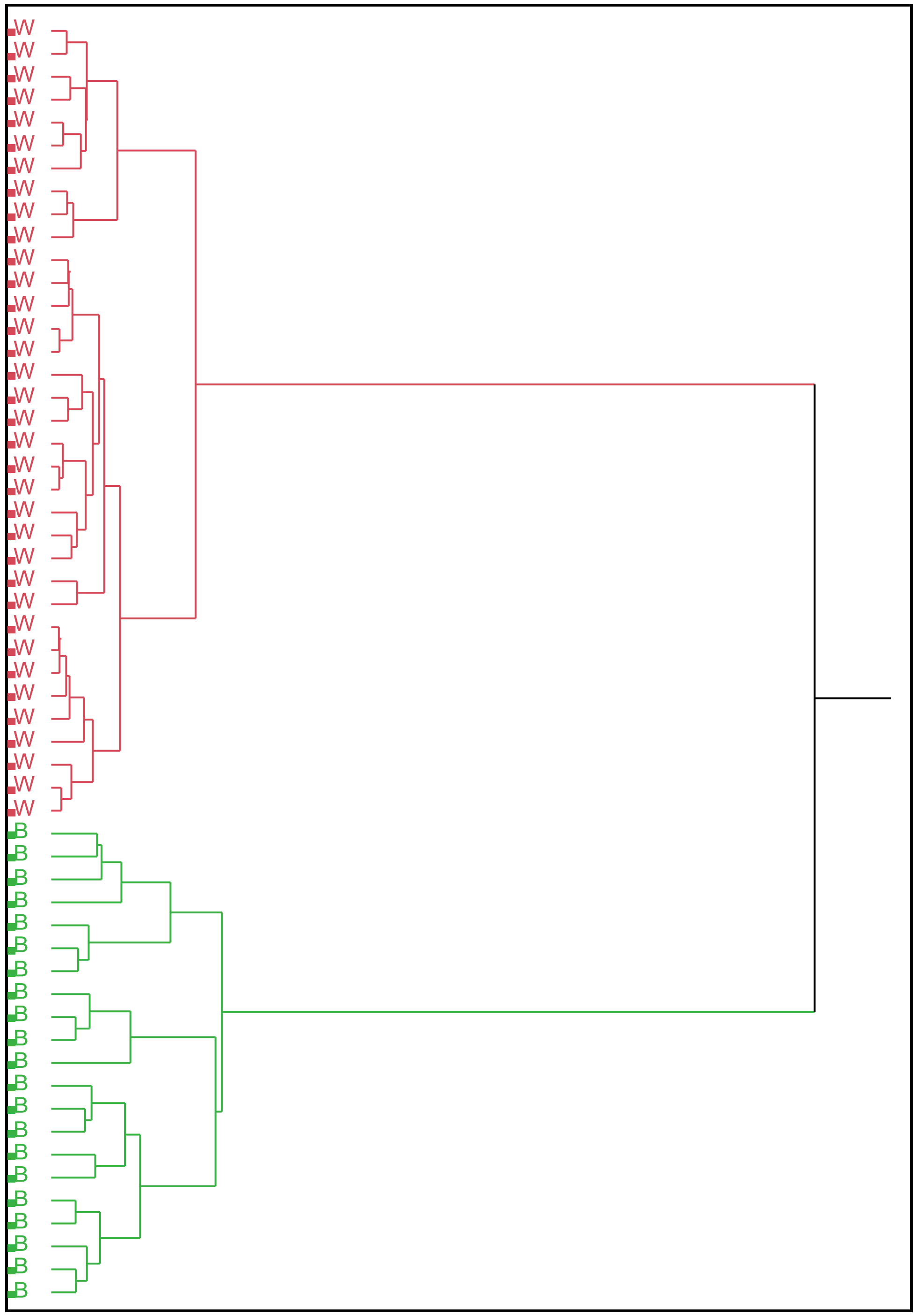

Figure 4.1: Ward's linkage cluster dendrogram (W denotes white rice and B denotes brown rice)

4.2 Principal Component Analysis

PCA analysis was performed on all the samples. PCA is a useful technique for transforming a large number of variables in a data set into a smaller and more coherent set of uncorrelated principal components (PCs) (Miller, JN. and Miller JC. 2005). Table 4.1 shows the eigenvalues. The first 2 PCs explains up to 87% of the variance and the each of the remaining components only contributes 4% or less.

Table 4.1: Eigenvalues

No	Eigenvalue	Percent	Cum Percent
1	6.4112	80.140	80.140
2	0.5765	7.207	87.347
3	0.3409	4.261	91.608
4	0.2849	3.562	95.170
5	0.1873	3.562	97.510
6	0.1062	1.328	98.838
7	0.0728	0.910	99.748
8	0.0202	0.252	100.000

4.2.1 Scree Plot

A scree plot also can be a useful visual aid for determining the appropriate number of PCs to be retained. The number of components depends on the "elbow" point at which the remaining eigenvalues are relatively small and all about the same size (Shiker, 2012). From Figure 4.2, such elbow point was noted at second components. This suggests extraction of only the first two PCs.

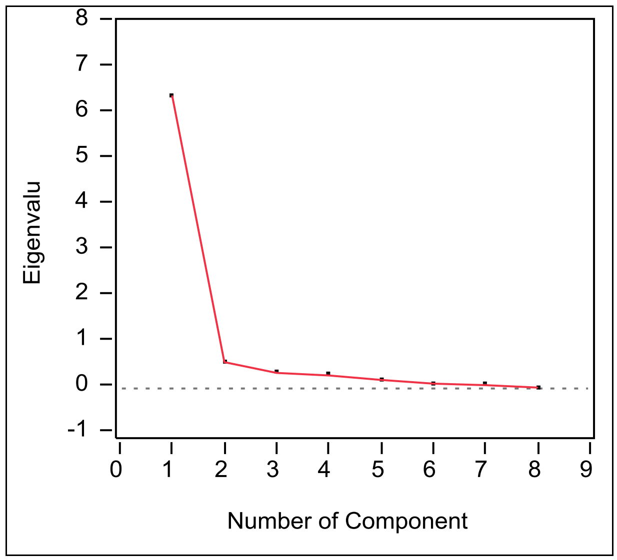

Figure 4.2: Scree plot

4.2.2 The Score Plot

The score plot gives an overview on how the samples are related to each other (Beebe *et al.*, 1998). It may reveal groupings of samples (clusters), trends and outliers (deviating samples). Usually, similar samples are close to each other on a given score plot (Saito *et al.*, 2006). Figure 4.3 displays the score plot for all samples.

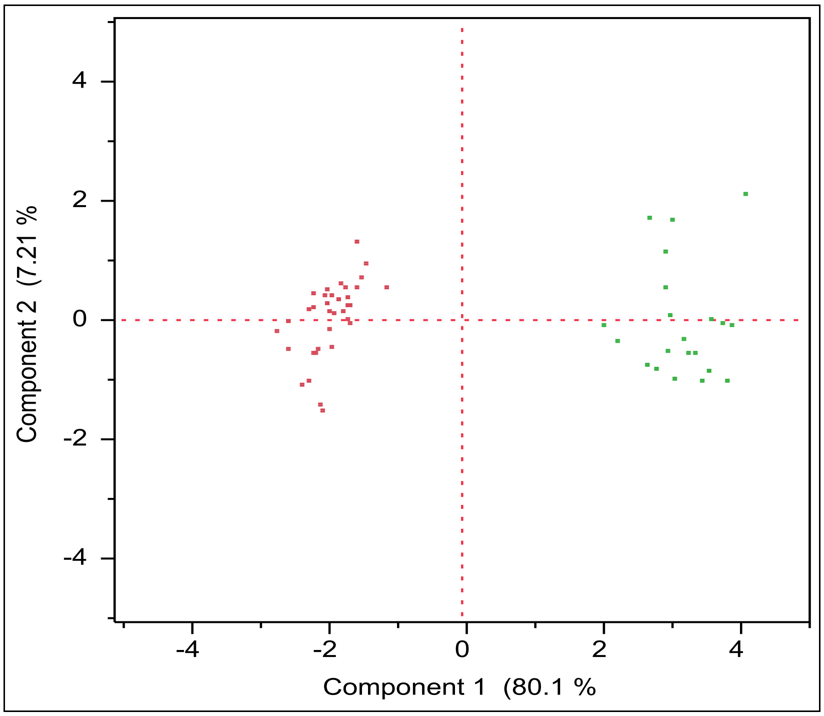

Figure 4.3: Score plot

The green dots represent brown rice samples whereas the red dots represent white rice samples. Both type of samples show distinctive grouping nature. Brown rice samples are all grouped together and have a heavy positive loading for PC1. The white rice samples are all grouped together and have a heavy negative loading for PC1. This was consistent with the HCA results.

4.2.3 The Loading Plot

For a given factor, the absolute magnitude of the loading for a measurement variable represents the relative contribution of the variables to the model and may be expected to display similar features with the raw data plot (Beebe *et al*., 1998). Loading Plot reveals the relationships between variables in the space of the first two components as shown in Figure 4.4. All variables showed positive loadings for PC1. This means those samples associated with relatively higher mineral content yield positive scores on PC1.

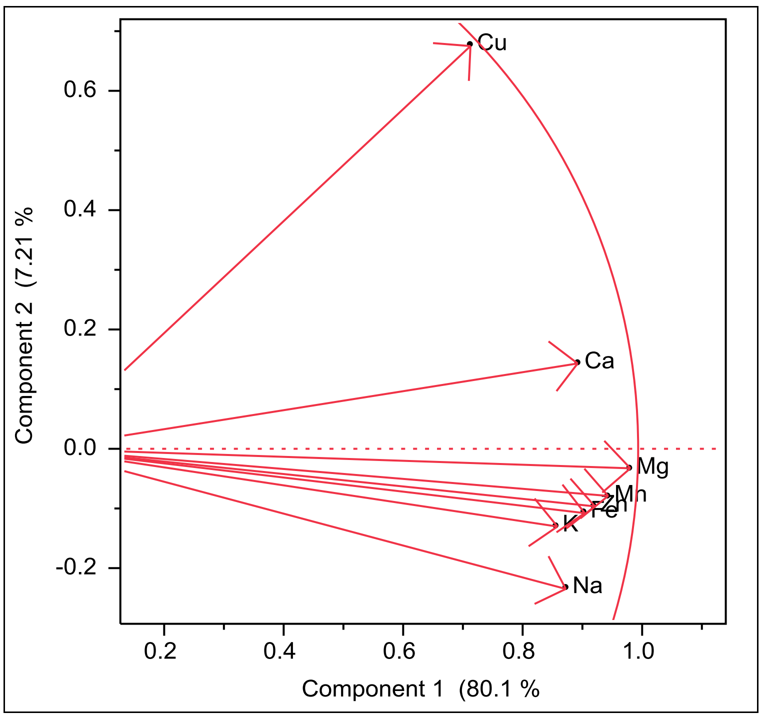

Figure 4.4: Loading plot

4.2.4 The Biplot

Biplot demonstrates the association between score plot (samples) and loading plot (variables) graphically (la Grange *et al.*, 2009).

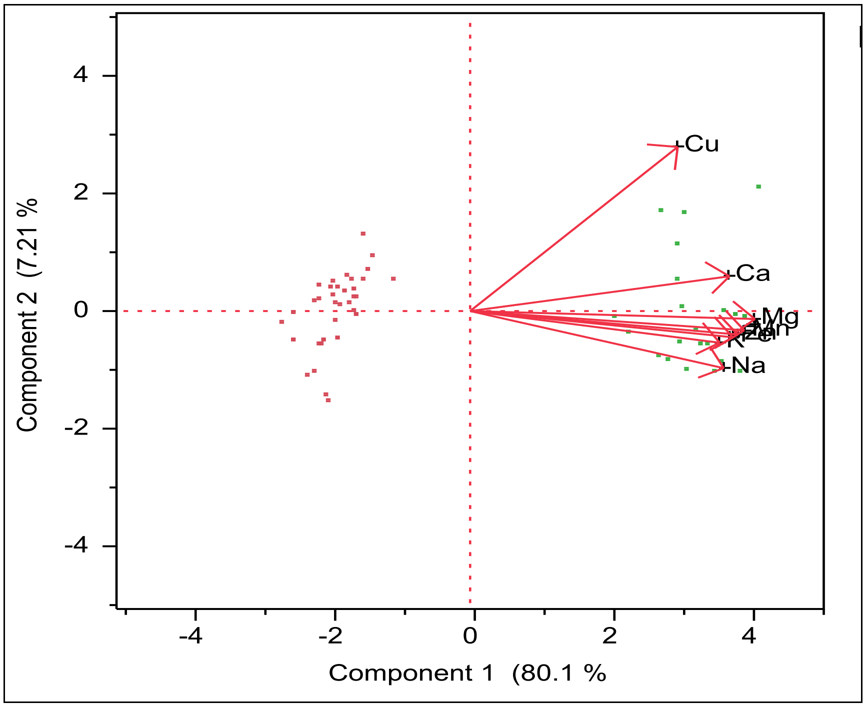

Figure 4.5: Biplot

In the Figure 4.5, it is noted that all the minerals and brown rice samples are mapped on the area with positive loading in PC1. In other words, brown rice are associated with higher mineral concentrations. This result is consistent with the literature finding where brown rice are generally rich in mineral compared to white rice.

4.3 Evaluation of Minerals Concentration in Brown Rice and White Rice

Based on the above mentioned analyses, there are differences between the pattern of minerals concentrations in brown rice and white rice. For details, Table 4.2 shows [1]the tabulated results obtained from ICP-MS analysis.

Table 4.2: Minerals concentration in brown rice and white rice

Type	Concentration (µg/g)							
	Na	Mg	K	Ca	Mn	Fe	Cu	Zn
White rice	1.5 ± 0.1	$(1 \pm 0.1)\times10^2$	$(3.6 \pm 0.2)\times10^2$	4.7 ± 0.3	4.4 ± 0.3	3.3 ± 0.2	0.7 ± 0.1	9.8 ± 0.6
Brown rice	3.6 ± 0.4	$(8.7 \pm 0.2)\times10^2$	$(1.4 \pm 0.2)\times10^3$	9.3 ± 0.8	9.7 ± 0.3	7.3 ± 0.5	1.0 ± 0.1	23.8 ± 2.2

Based on ANOVA, minerals concentrations are different between brown rice and white rice. This finding again confirms the trends revealed by HCA and PCA. In general, the mean concentrations of macro minerals follow the sequence K>Mg>Ca>Na for both brown rice and white rice. Meanwhile the concentrations of micro minerals follow the sequence Zn>Mn>Fe>Cu for both brown rice and white rice. K has the highest mineral concentration in both brown rice and white rice while Cu has the least mineral concentration in both types of rice.

[1] All results are reported as mean ± 95% confidence intervals, in µg/g

Thus results found demonstrated that the mineral content in brown rice is always greater than in the white rice. This can be explained considering that the production of brown rice involves only the removal of the shell that covers the grain in its natural state. Initially, the mature rice grain is harvested as a covered grain (paddy), with the caryopsis enclosed in a tough siliceous hull or husk (Juliano and Bechtel 1985). The husk, seed, coat, nucellus, aleurone, endosperm and germ are the principle component of paddy seed (Bryant *et al*., 2005). Milling separates the husk to produce brown rice. Brown rice is the unmilled rice containing the outer grain layers such as pericarp, the seed coat and nucellus, the germ or embryo and the endosperm (Ajimilah and Rosniyana 1995). The outer grain layers are much denser in minerals than the inner parts, causing the retention of most minerals contributing to high nutritional value for brown rice.

Previous studies have shown a relatively large proportion of Fe, Mg, K and Mn is associated with the outer grain layers and these elements are rapidly lost during polishing (Lamberts *et al*., 2007). In some cases, much higher mean concentration of Zn compare to Fe is due to greater proportion of Fe lost by polishing than is the case for Zn. This reflects their distribution pattern within the grain, Fe being mainly confined to the outer grain layers while Zn is more evenly distributed (Greffeuille *et al*., 2011). In this study, however the lost of Zn and Fe is about the same. Some mineral such as K and Mg values decreased with successive milling fractions (Luh *et al*., 1991).These studies generally show decreased concentrations of macronutrients moving from the outer edge to the centre of the grain, as is also the case for micronutrients, (Hansen, 2012). A longitudinal grain section is shown in Figure 4.6.

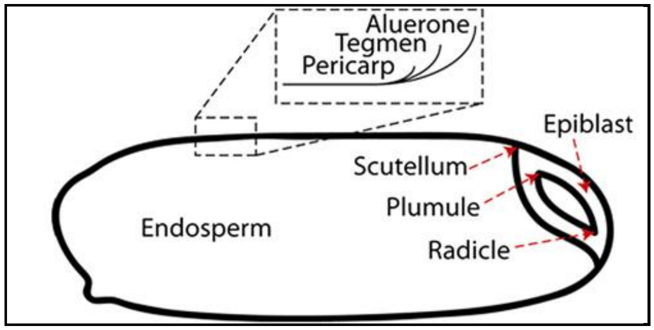

Figure 4.6: Longitudinal grain section
Source: (Hansen, 2012)

For the white rice, the production process of this involves a polishing step of crude rice in order to remove all outer layers, including the pericarp, testa and embryo (Ogiyama *et al*., 2008). Although polishing improves the appearance and taste of rice, this operation causes a loss of nutritional value for the rice, because some substances of high nutritional value contained in the embryo are removed. This explains the results achieved in Table 4.2.

4.3.1 Sodium

Statistical significance of the data obtained was analyzed by t-test. Observed significance probabilities of 0.05 or less are considered evidence to check whether there is any significant different between the two means or whether the difference might have occurred by chance (Miller, JN. & Miller JC., 2005). In Figure 4.7, the mean line across the middle of each diamond represents the group mean while the top and bottom of each diamond represent the confidence interval for each group. Overlap marks appear as lines above and below the group mean. B indicates brown rice samples, and W indicates white rice samples. When overlap marks in one diamond that are closer to the mean of another diamond than that diamond's overlap marks indicate that those two groups are not significantly different at the given confidence level. Based on above criteria, Figure 4.7 shows that mean Na concentration in brown rice significantly higher than white rice at (P< 0.001). Thus intake of brown rice provides consumers with higher content of Na mineral compared with white rice although generally Na concentration in rice is low (Antoine *et al*., 2012).

Na is essential to humans, used for regulating blood pressure and blood volume, and critical for muscle and nerve function (Antoine *et al*., 2012). Na acts as a transporter of nutrients in and out of cells, works to promote healthy cell development and regulation. Working in perfect synch with K, Na also helps to regulate fluid levels around cells.

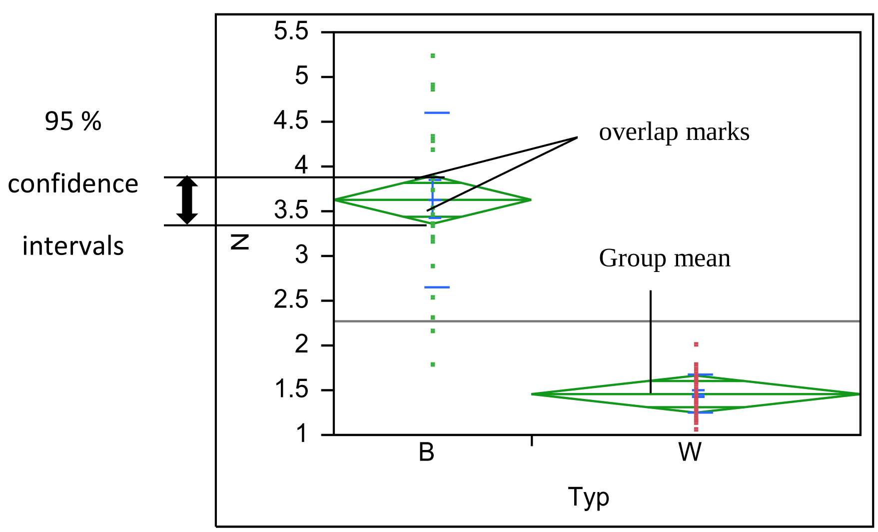

Figure 4.7: t-test for Na

4.3.2 Magnesium

Figure 4.8 shows the mean concentration of Mg in brown rice is significantly higher than in white rice (*P*<0.001). Mg is an essential macronutrient required for an extensive range of metabolic, regulatory, and structural activity. Mg deficiency is associated with increased risk of many age related diseases, including cardiovascular disease, hypertension, diabetes, osteoporosis, and some cancers.

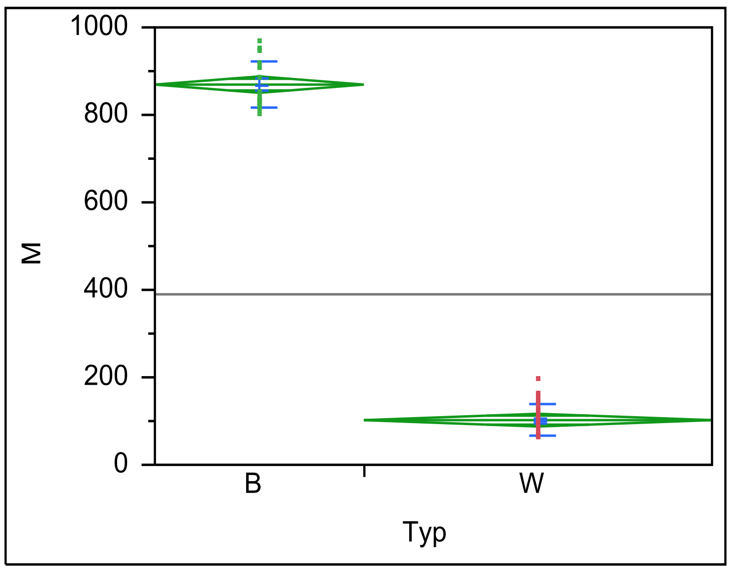

Figure 4.8: t-test for Mg

4.3.3 Potassium

Figure 4.9 shows the mean concentration of K in brown rice is significantly higher than in white rice (*P*<0.001). K is the third most abundant mineral in the human body and is essential to human life. K is the dominant intracellular cation and functions as a regulator in the acid–base and the osmotic balance of the cell (McDowell, 1992).

K is also known as a cofactor in many enzymes involved in protein synthesis, energy transfer and the storage of carbohydrates for use as fuel in muscles (Schmidt-Nielsen, 1997) The pivotal role of K in cardiovascular disease is by helping to maintain the heart's regular beat and make sure blood pressure levels remain even. K also plays an essential role in providing nutrients to cells, and in addition, K works to maintain balanced fluid levels in and around each cell that exists within the human body.

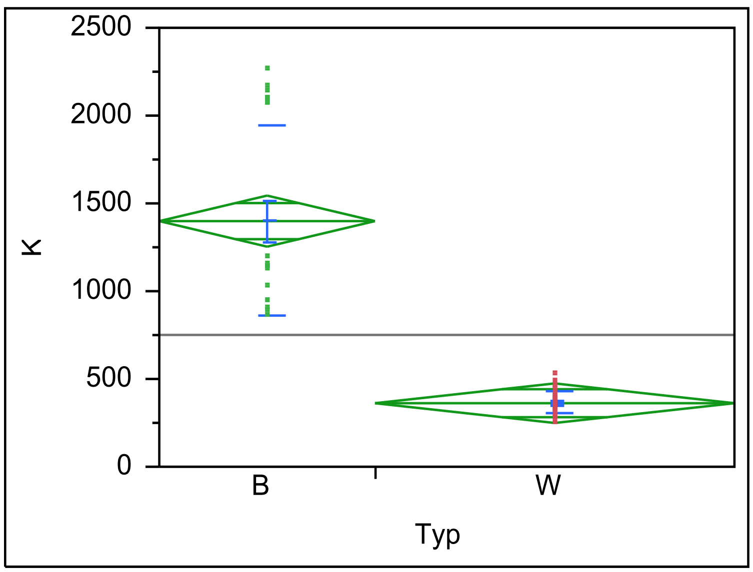

Figure 4.9: t-test for K

4.3.4 Calcium

Figure 4.10 showed the mean concentration of Ca in brown rice is significantly higher than in white rice (P<0.001). Ca is the most abundant mineral in the human body and is stored mostly in the bones and teeth. It is also essential for muscle contraction, nervous system function, blood vessel expansion and contraction, and secretion of hormones and enzymes

(McDowell, 1992). Ca may prevent human colorectal carcinoma and myeloma (Katzel *et al*., 2007). The optimum Ca intake for the human body has been estimated to be from 800 (<5 years age) to 1,500 (>65 years age) mg/day (Kim *et al*., 2003).

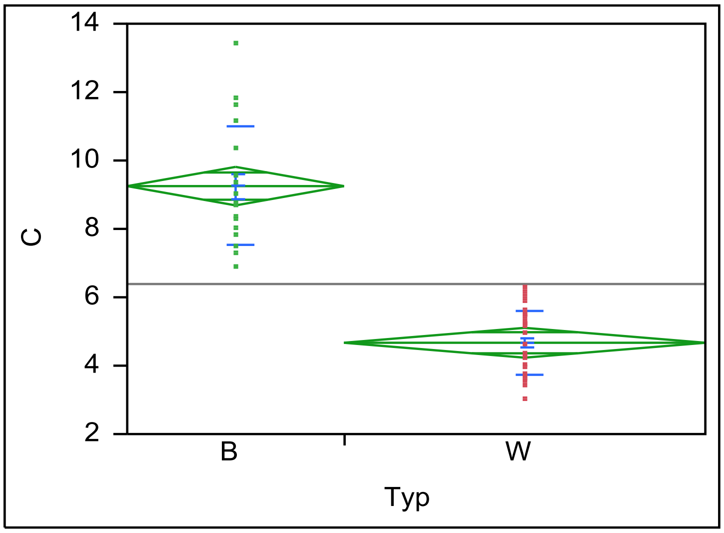

Figure 4.10: t-test for Ca

4.3.5 Manganese

Figure 4.11 shows the mean concentration of Mn in brown rice is significantly higher than in white rice (*P*<0.001). Brown rice is known as a good source of Mn (Crossgrove and Zheng, 2004). Mn is essential to human beings. It can function as an enzyme activator for several enzymes and as a component of some metalloenzymes (McDowell, 1992). It is also important for bone growth, carbohydrate and lipid metabolism. Mn is also required for normal amino acid, lipid, protein, and carbohydrate metabolism.

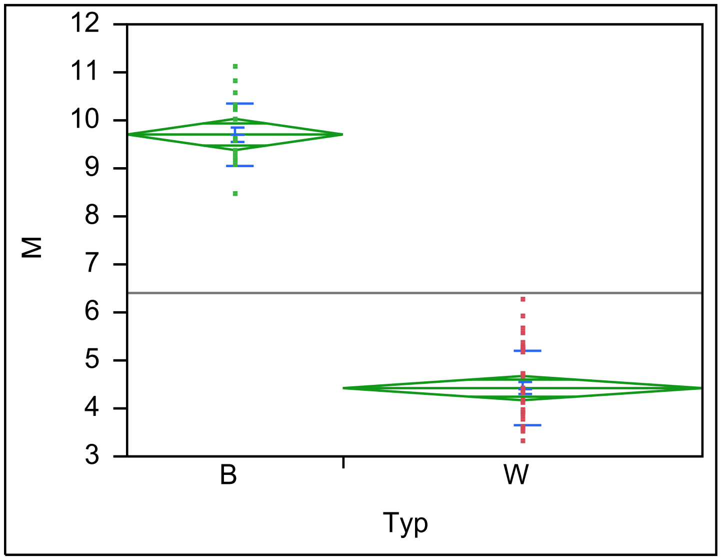

Figure 4.11: t-test for Mn

4.3.6 Iron

Figure 4.12 shows the mean concentration of Fe in brown rice which is significantly higher than in white rice (*P*<0.001). The WHO lists Fe deficiency as the 6th leading cause of illness and disease in low income countries (Hotz and Brown, 2004). Nearly 3.7 billion people are Fe deficient, with two billion of these so severely deficient they can be described as anemic (WHO, 2009). There are several recognized factors related to this deficiency. Low bioavailability of Fe in foods, inability to meet Fe demand during growth spurts such as infancy and pregnancy, blood loss in women of childbearing age through menstruation and childbirth, blood losses from parasitic infections and loss from diseases like malaria (McDowell, 1992). Most of the Fe in the body is found in hemoglobin and myoglobin, both involved in oxygen transport. The amount of bioavailable Fe in rice is low (Glahn *et al.*, 2002). This is a likely contributor to the global Fe deficiency problem. The polishing or milling process may remove the remaining Fe minerals and enrichment may be an important tool in combating Fe deficiency (Liang, 2007).

The past decade has seen growing interest in developing varieties of staple grain crops with enhanced concentrations of elements Fe to improve the nutritional quality of grain for human consumption (White and Broadley 2005; Cakmak, 2008; McDonald *et al*., 2008; Wissuwa *et al*., 2008).

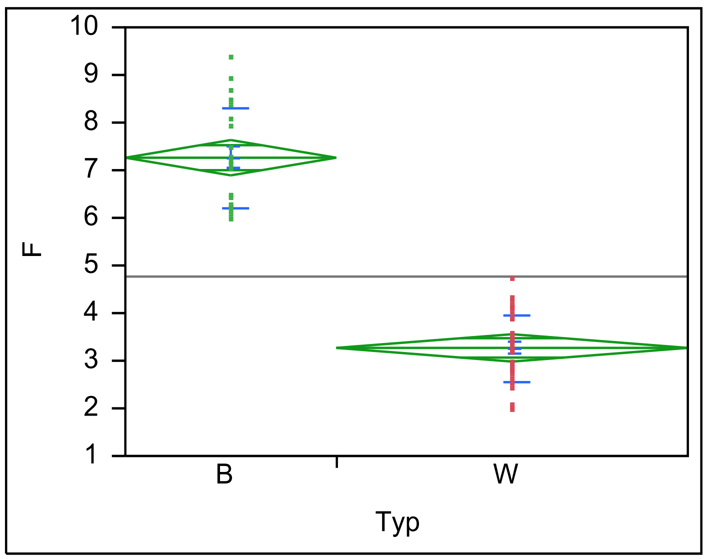

Figure 4.12: t-test for Fe

4.3.7 Copper

Figure 4.13 shows the mean concentration of Cu in brown rice is significantly higher than in white rice (*P*<0.001). Cu is essential to humans in small quantities as a key component of redox enzymes and hemocyanin. It is involved in mitochondrial function, cellular metabolism, connective tissue formation, and the absorption, storage and metabolism of iron (McDowell, 1992). Cu also plays a significant role in central nervous system development. Low concentrations of Cu may result in incomplete development (Desai and Kaler, 2008).

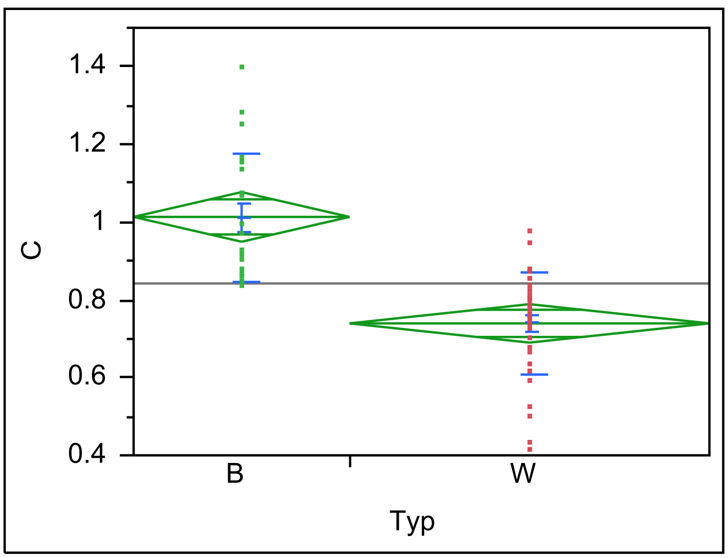

Figure 4.13: t-test for Cu

4.3.8 Zinc

Figure 4.14 shows the mean concentration of Zn in brown rice is significantly higher than in white rice (*P*<0.001). It is likely that the relatively low concentrations in rice are a function of Zn deficient soils or soils with properties that make this element biologically unavailable for uptake by the plant. Zn deficiencies affect over three billion people worldwide, and it has been reported that 50% of soils cultivated with cereals are low in bioavailable Zn (Cakmak, 2008).

The WHO lists Zn deficiency as the 5th leading cause of illness and disease in low income countries (Hotz and Brown, 2004). A Zn deficiency in the human body reduces the serum testosterone level, which is linked to oligospermia, a severe immune dysfunction mainly affecting T helper cells hyperammonemia, neurosensory disorders, and decreased lean body mass (McDowell, 1992; Prasad, 2008).

The past decade has also seen growing interest in developing varieties of staple grain crops with enhanced concentrations of elements Zn to improve the nutritional quality of grain for human consumption (White and Broadley 2005; Cakmak, 2008; McDonald *et al*., 2008; Wissuwa *et al*., 2008).

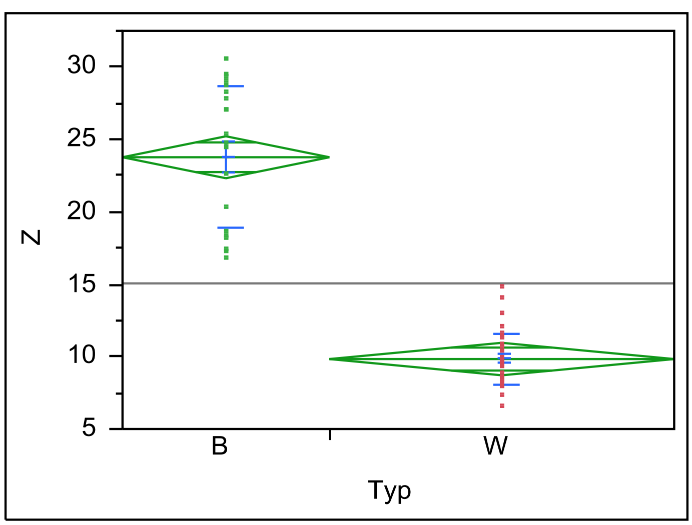

Figure 4.14: t-test for Zn

4.4 Contribution to Recommended Daily Intake (RDI)

The dietary mineral intake levels for Malaysians were estimated based on the results obtained in this study and the assumption that the average rice consumption per capita at 203 g per day (FAOSTAT, 2009). Both estimated % RDIs for adults (aged 19 to 50 for both male and female) and the RDIs by United States Department of Agriculture (USDA, 2010) are shown in Table 4.3 and Table 4.4.

Table 4.3: Daily intake estimates of Malaysian brown rice with RDI values

Mineral	Mean (µg/g)	Daily Intake (mg/day)	RDI, mg/day (Male)	RDI, mg/day (Female)	% of RDI (Male)	% of RDI (Female)
Ca	9.3	1.9	1000	1300	0.2	0.2
Cu	1.0	0.2	0.9	0.9	22.2	22.2
Fe	7.3	1.5	8	18	18.8	8.3
K	1400	284.2	4700	4700	6.0	6.0
Mg	870	176.6	420	320	42	55.2
Mn	9.7	2.0	2.3	1.8	87	111
Na	3.6	0.7	2300	2300	0.03	0.03
Zn	23.8	4.8	11	8	43.6	60

Source: (USDA, 2010)

Table 4.4: Daily intake estimates of Malaysian white rice with RDI values

Mineral	Mean (µg/g)	Daily Intake (mg/day)	RDI, mg/day (Male)	RDI, mg/day (Female)	% of RDI (Male)	% of RDI (Female)
Ca	4.7	1.0	1000	1300	0.1	0.1
Cu	0.7	0.1	0.9	0.9	11.1	11.1
Fe	3.3	0.7	8	18	8.8	3.9
K	360	73	4700	4700	1.6	1.6
Mg	100	20.3	420	320	4.8	6.3
Mn	4.4	0.9	2.3	1.8	39.1	50
Na	1.5	0.3	2300	2300	0.01	0.01
Zn	9.8	2.0	11	8	18.2	25

Source: (USDA, 2010)

Overall brown rice showed a higher mineral contribution to the RDI compared to white rice for all minerals observed in this study. Brown rice is a significant source of Mn with the average intake accounting for 87% of the RDI for male and 111% of the RDI for female. In white rice the average intake accounted for 39.1% of the RDI for male and 50% of the RDI for male. In other words rice appears to be a significant source of Mn for the Malaysian population. Although the upper limit established is 11 mg daily (HHS, 2010) for adults it bears noting that the Malaysian diet has other significant sources of Mn including oats and leafy vegetables and although toxicity by dietary means is still unlikely, deficiency does not appear to be an issue (Tee *et al*., 1997). Mn helps produce energy from protein and carbohydrates and is involved in the synthesis of fatty acids, which are important for a healthy nervous system, and in the production of cholesterol, which is used by the body to produce sex hormones (Fortin, 1996)

Other nutritionally important minerals in brown rice which significantly contribute in a single portion are Mg, Zn, and Cu whose values fall within the range of 22 % to 60 % of the RDI. Zn deficiencies have been well described in literature and the WHO lists these as leading causes of illness and disease in majority countries (Hotz and Brown, 2004). Brown rice provides 60 % for female and 43.6 % for male of the RDI values for Zn. In comparison white rice only accounts for 25 % in female and 18.2 % in male of the RDI values for Zn. Cu is found in a variety of foods (Tee *et al*., 1997) and its relatively low RDI values of 0.9 mg/day for both male and female means that deficiency is rare. Deficiency may be as a result of interfering factors affecting bioavailability such as Fe deficiency, molybdenum and excessive Zn ingestion (Kumar, 2007).

On the other hand, the mineral K present in highest concentration in both brown rice and white rice only reached 6% and 1.6% of the RDI. Therefore, although rice is a rich source of K with brown rice contributing the most to the RDI at 6 % its worth mentioning that intake of foods rich in K must be taken besides rice as the RDI values for K is the highest at 4700 mg/day given the importance of K in human body. The contribution of Na to dietary intake is insignificant, with brown rice contributing 0.03 % of the RDI and white rice contributing 0.01 % of the RDI. Although the RDI values for Na is rather high at 2300 mg/day, it does not cause a great concern as there is an excess of Na in modern diet (Tee *et al.*, 1997).

Rice does not contribute significantly to the daily dietary intake of Ca. The estimate shows Malaysians getting 0.2 % of the suggested RDI values by consuming brown rice and 0.1 % of the RDI by consuming white rice. Since Ca is the most abundant mineral in the human body and is stored mostly in the bones and teeth (McDowell, 1992), the intake of foods rich in Ca is necessary. The RDI values for Fe is significantly different for males and females, thus, while males may obtain as much as 18.8% of their RDI from brown rice and 8.8% from white rice the contribution to RDI for females is, at best, only 8.3% from brown rice and 3.9% from white rice.

4.5 Potential of Brown Rice As a Nutritionally Enhanced Rice

Over three billion people in the world are mineral malnourished, because of their sustenance on staple crops. Among all the important staple crops, rice has the highest food and food energy yield in Asia, but plays a secondary role to the potato in western diets. The mineral elements contained in rice are beneficial to plant growth and human health and their concentrations in grain are linked to genetic factors and environmental conditions.

Rice is an indispensable staple food for half of the world's population. In countries where rice is used as a staple food, the per capita consumption is very high, ranging from 62 to 190 kg/year (Lu *et al*., 2008), thus even a small increase in the nutritive value of rice can be highly significant for human nutrition. From this study, it is evident that brown rice contains higher minerals content and a higher contribution to RDI values, thus it is agreeable that brown rice possess the potential of a nutritionally enhanced rice.

4.6 Mineral Contents in Different Brands of Malaysian Brown Rice and White Rice

The results for mineral concentration in each brand of brown rice and white rice obtained from ICP-MS analysis are tabulated and shown in Table 4.5 below. All results are reported as mean ± 95% confidence intervals and n=7.

Table 4.5: Minerals concentration in each brand of brown rice and white rice

Brand	(µg/g)							
	Na	Mg	K	Ca	Mn	Fe	Cu	Zn
WRA	1.4±0.1	66±4	436±61	4.8±0.8	5.3±0.3	3.1±0.5	0.7±0.1	9.5±0.6
WRB	1.4±0.2	70.3±7	382±42	4.4±0.7	5.1±0.6	3.7±0.6	0.5±0.1	9.8±0.9
WRC	1.3±0.2	156±16	375±18	4.4±1	4.2±0.3	3.3±0.8	0.9±0.1	10.9±2
WRD	1.5±0.1	104±19	333±16	4.4±0.8	3.9±0.2	3.3±0.8	0.8±0.1	10.2±2
WRE	1.7±0.2	114±6	284±16	5.3±0.8	3.6±0.2	2.9±0.5	0.8±0.1	8.8±2
BRA	3.7±1	935±21	2128±64	9.4±2	9.4±0.8	6.9±1.3	1±0.1	24.6±5
BRB	3±0.8	844±20	1154±26	9.5±2	10±0.4	7±0.7	1.2±0.1	21±5
BRC	4.1±0.6	829±25	915±50	8.8±1.1	9.7±0.7	7.8±0.7	0.9±0.1	26±4

The evaluation of the data obtained from the brown rice and white rice samples revealed that there is slight difference in the mineral compositions among the rice brands analyzed in this study. Overall all 3 brands for brown rice contributed to a higher content of mineral when compared to all 5 brands of white rice. These differences can be produced by slight differences in rice processing of each brand, or due to their different origins (Luh *et al.*, 1991).

Also, the chemical and nutritional quality of rice grain varies considerably and this may be attributed to genetic factors, environmental influences, fertilizer treatments, degree of milling and storage conditions (Roy *et al*., 2011). Brown rice brands are BRA, BRB and BRC meanwhile white rice bands are WRA, WRB, WRC, WRD and WRE respectively.

4.7 Comparison of Previous Literature with Current Study

Results from the current study was compared with previous literature involving data from different countries such as Jamaica, Brazil and China. Table 4.6 shows the comparison of mineral composition in brown rice and Table 4.7 shows the comparison of mineral composition in white rice. The results were calculated to (μg/g).

Table 4.6: Mineral composition of brown rice from current study and various literature data calculated to (μg/g)

(μg/g)	Ref.			
	Current study	Antoine *et al*., 2012	Heinemann *et al*., 2005	Jiang *et al*., 2008
Na	3.6 ± 0.4	15.1 ± 13.2	5.4 ± 2	39.1 ± 18.5
Mg	$(8.7\pm 0.2)\times10^{2}$	$(1.2\pm 0.3)\times10^{3}$	168.8 ± 5.7	$(1.4\pm 0.2)\times10^{3}$
K	$(1.4\pm0.2)\times10^{3}$	$(2.2\pm 0.6)\times10^{3}$	1817.1 ± 92.7	$(3.4\pm 0.5)\times10^{3}$
Ca	9.3±0.8	$(1.0\pm 0.4)\times10^{2}$	68.5 ± 4.3	$(2\pm 0.7)\times10^{2}$
Mn	9.7 ± 0.3	26.5 ± 12.2	3.6 ± 0.5	22.8 ± 6.8
Fe	7.3 ± 0.5	20.1 ± 7.8	5.7 ± 3.5	23.1 ± 7.6
Cu	1.0 ± 0.1	3.0 ± 1.1	1.6 ± 0.7	10.1 ± 4.1
Zn	23.8 ± 2.2	20.2 ± 2.7	19.8 ± 1.1	34.7 ± 5.4

Table 4.7: Mineral composition of white rice from current study and various literature data calculated to (μg/g)

(μg/g)	Ref.			
	Current study	Antoine *et al*., 2012	Heinemann *et al*., 2005	Jiang *et al*., 2008
Na	1.5 ± 0.1	6.0 ± 3	5.3 ± 0.6	26.6 ± 16.4
Mg	$(1\pm 0.1)\times10^2$	$(3.7\pm1.3)\times10^2$	$(1.5\pm0.2)\times10^2$	$(2.1\pm0.7)\times10^2$
K	$(3.6\pm0.2)\times10^2$	$(9.1\pm3.9)\times10^2$	$(6.6\pm0.6)\times10^2$	$(8.7\pm2.1)\times10^2$
Ca	4.7 ± 0.3	$(1.3\pm 1.4)\times10^2$	67 ± 4.2	$(1.2\pm0.5)\times10^2$
Mn	4.4 ± 0.3	10.5 ± 3.7	4.5 ± 0.6	9 ± 2
Fe	3.3 ± 0.2	22.3 ± 37.9	4.0 ± 2.9	5.1 ± 3
Cu	0.7 ± 0.1	1.7 ± 0.6	1.8 ± 0.4	7.5 ± 2.7
Zn	9.8 ± 0.6	15.6 ± 1.9	20.9 ± 0.9	26.8 ±3.9

An overview of the Table 4.6 and Table 4.7 highlighted that the Ca content of Malaysian brown rice and white rice was generally low, with a mean concentration of only 9.3 μg/g among the brown rice samples and 4.7 μg/g in the white rice. However, adequate intake of Ca for adults is 1000-1300 mg/day (HHS, 2010) and as such even the Jamaican, Brazillian and Chinese would not contribute significantly to dietary Ca intake. Thus there is a need in the intake of other food sources rich in Ca for all the consumers of the discussed countries along Malaysia.

With the exception of Ca, the other minerals presented concentrations similar to the range reported by current study although Malaysian brown rice and white rice contributed a lower concentration for most of the minerals compared to previous literatures. This could be due to the fact that mineral contents largely depend on geographical factor, availability of soil nutrients, agriculture practices, varietal differences and processing conditions (Welch, 2002).

CHAPTER 5

CONCLUSION

The PCA and HCA techniques showed that the mineral compositions of Malaysian brown rice and white rice samples are different. It is noteworthy that the brown rice analyzed contains on average, significantly greater concentrations of Na, Mg, K, Ca, Mn, Fe, Cu and Zn in comparison with the white rice samples. Although rice is considered to be the major staple consumed in Malaysia, but the actual contribution to mineral nutrition for most minerals is not significant referring to the RDI values, especially considering that the most widely consumed rice is white rice. Thus the intake of brown rice contributes a greater concentration of minerals although there is still a need for intake of other food sources rich in minerals to obtain a balanced diet.

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APPENDICES

Appendix A: Rice samples

<table>
<tr><th colspan="2">Type</th></tr>
<tr><th>Brown Rice</th><th>White rice</th></tr>
<tr><td>

BRA</td><td>

WRA</td></tr>
<tr><td>

BRB</td><td>

WRB</td></tr>
</table>

<table>
<tr>
<td rowspan="3">

BRC</td>
<td>

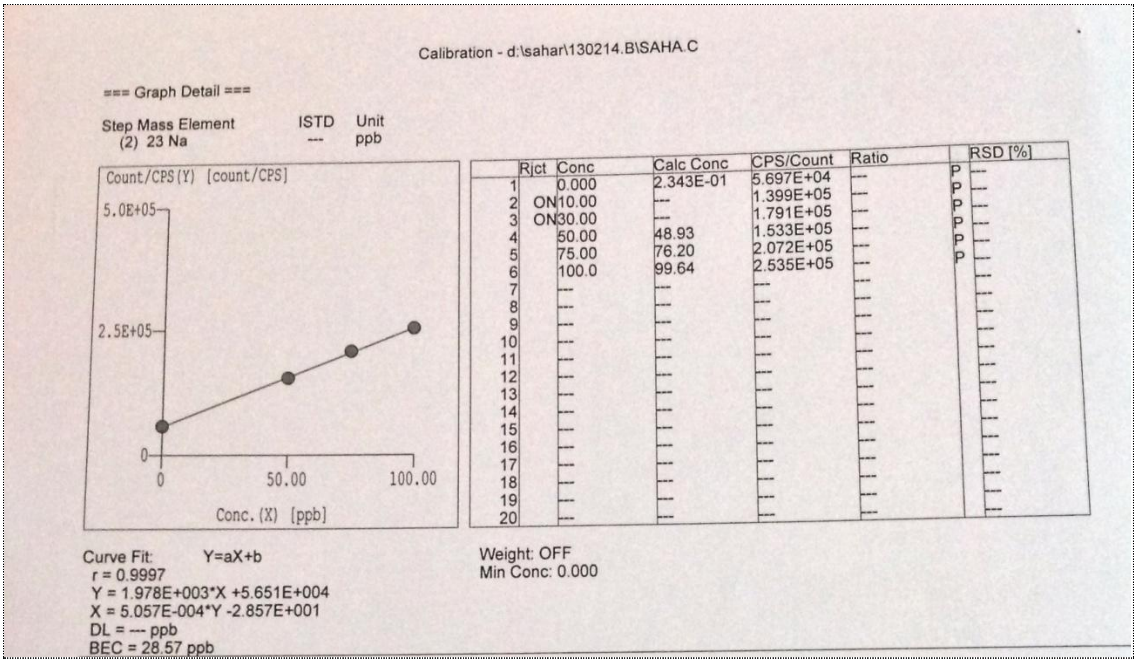
WRC</td>
</tr>
<tr>
<td>

WRD</td>
</tr>
<tr>
<td>

WRE</td>
</tr>
</table>

Appendix B: Calibration curve

Appendix B1: Calibration curve for Na


Calibration - d:\sahar\130214.B\SAHA.C

=== Graph Detail ===

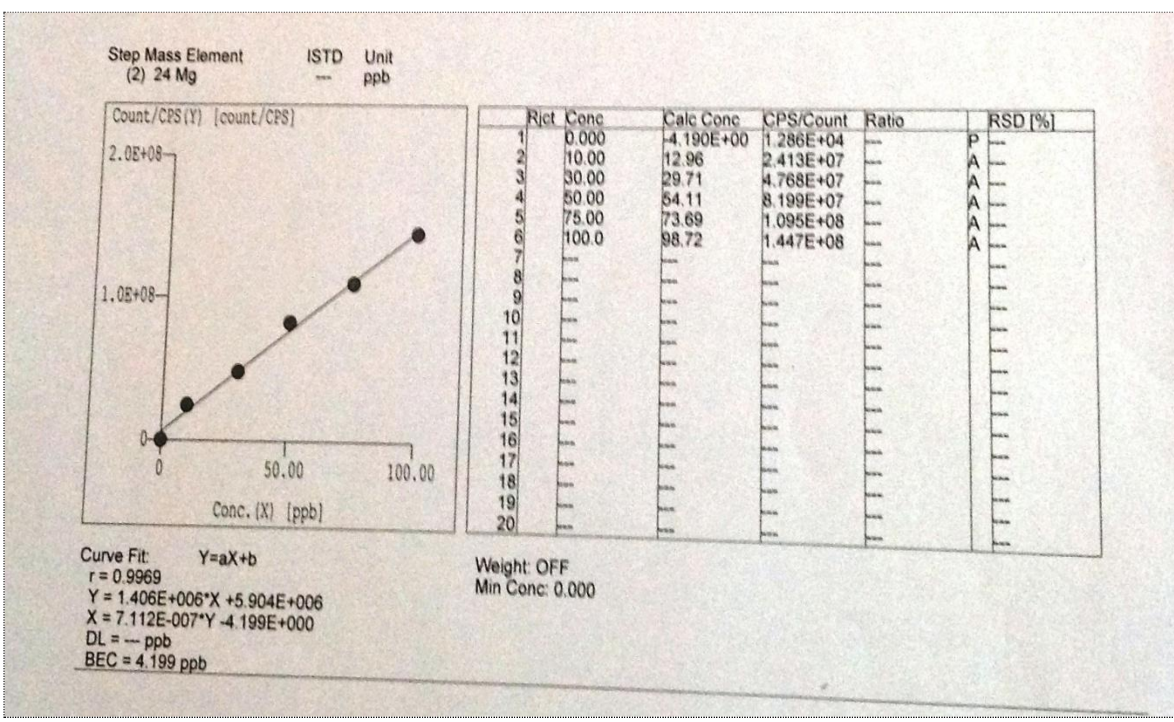
Step Mass Element ISTD Unit
(2) 23 Na --- ppb

	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	2.343E-01	5.697E+04	---	P	---
2	ON	10.00	---	1.399E+05	---	P	---
3	ON	30.00	---	1.791E+05	---	P	---
4		50.00	48.93	1.533E+05	---	P	---
5		75.00	76.20	2.072E+05	---	P	---
6		100.0	99.64	2.535E+05	---	P	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9997
Y = 1.978E+003*X +5.651E+004
X = 5.057E-004*Y -2.857E+001
DL = --- ppb
BEC = 28.57 ppb

Weight: OFF
Min Conc: 0.000

Appendix B2: Calibration curve for Mg


Step Mass Element ISTD Unit
(2) 24 Mg --- ppb

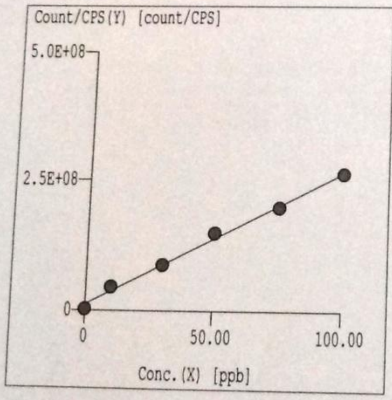
	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-4.190E+00	1.286E+04	---	P	---
2		10.00	12.96	2.413E+07	---	A	---
3		30.00	29.71	4.768E+07	---	A	---
4		50.00	54.11	8.199E+07	---	A	---
5		75.00	73.69	1.095E+08	---	A	---
6		100.0	98.72	1.447E+08	---	A	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9969
Y = 1.406E+006*X +5.904E+006
X = 7.112E-007*Y -4.199E+000
DL = --- ppb
BEC = 4.199 ppb

Weight: OFF
Min Conc: 0.000

Appendix B3: Calibration curve for K

Step Mass Element ISTD Unit
(2) 39 K --- ppb



	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-3.831E+00	1.970E+06	---	A	---
2		10.00	12.91	4.415E+07	---	A	---
3		30.00	29.60	8.618E+07	---	A	---
4		50.00	53.83	1.472E+08	---	A	---
5		75.00	73.29	1.962E+08	---	A	---
6		100.0	99.20	2.615E+08	---	A	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9972
Y = 2.519E+006*X +1.162E+007
X = 3.970E-007*Y -4.613E+000
DL = --- ppb
BEC = 4.613 ppb

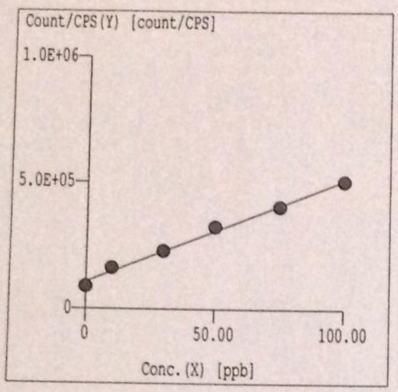
Weight: OFF
Min Conc: 0.000

Appendix B4: Calibration curve for Ca

Calibration - d:\sahar\22101301.B\SHARIFAH.C

=== Graph Detail ===

Step Mass Element ISTD Unit
(2) 43 Ca --- ppb



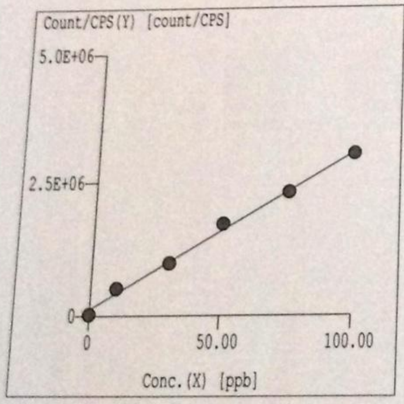
	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-4.654E+00	8.725E+04	---	P	---
2		10.00	13.34	1.603E+05	---	P	---
3		30.00	30.05	2.281E+05	---	P	---
4		50.00	53.87	3.247E+05	---	P	---
5		75.00	73.72	4.053E+05	---	P	---
6		100.0	98.68	5.066E+05	---	P	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9966
Y = 4.058E+003*X +1.061E+005
X = 2.464E-004*Y -2.616E+001
DL = --- ppb
BEC = 26.16 ppb

Weight: OFF
Min Conc: 0.000

Appendix B5: Calibration curve for Mn

Step Mass Element (2) 55 Mn ISTD --- Unit ppb



	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-3.413E+00	2.154E+04	---	P	---
2		10.00	12.90	4.991E+05	---	P	---
3		30.00	28.79	9.646E+05	---	P	---
4		50.00	53.79	1.696E+06	---	A	---
5		75.00	74.04	2.290E+06	---	A	---
6		100.0	98.90	3.018E+06	---	A	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9975
Y = 2.928E+004*X +1.215E+005
X = 3.415E-005*Y -4.148E+000
DL = --- ppb
BEC = 4.148 ppb

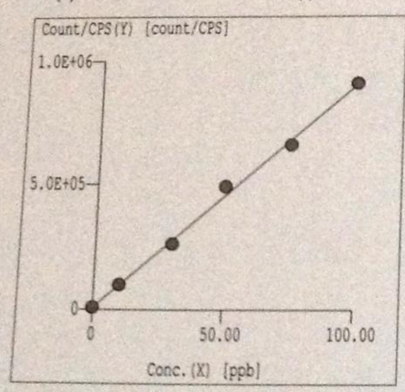
Weight: OFF
Min Conc: 0.000

Appendix B6: Calibration curve for Fe

Calibration - d:\sahar\130214.B\SAHA.C

=== Graph Detail ===

Step Mass Element (2) 56 Fe ISTD --- Unit ppb

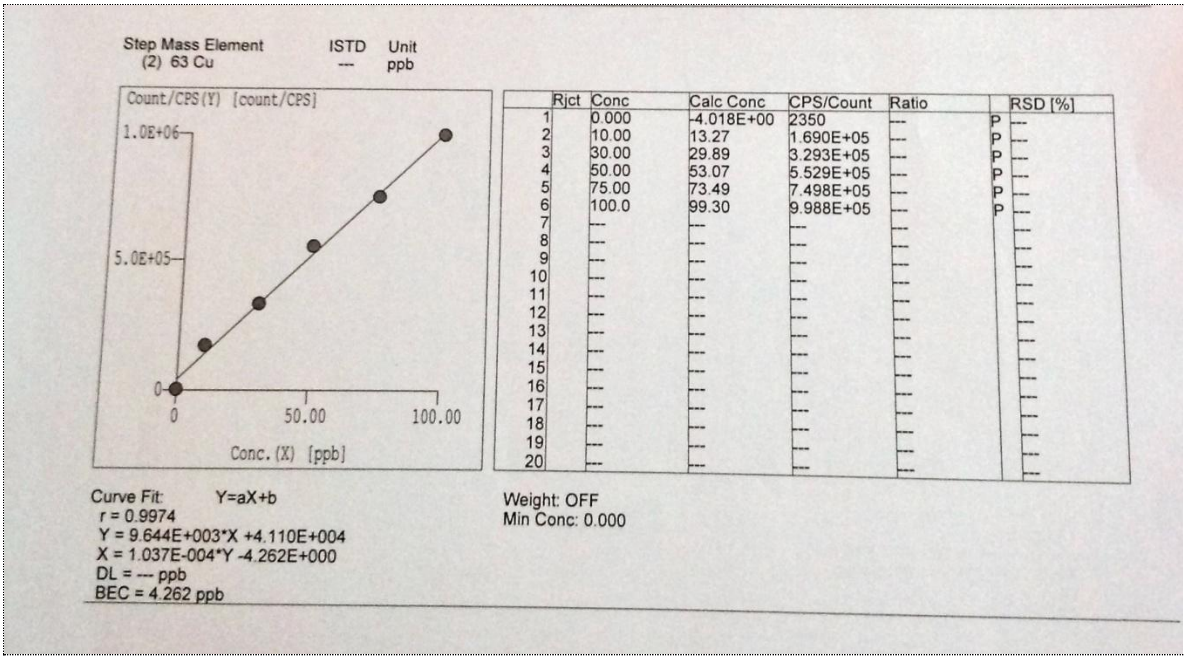


	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-1.008E-01	7826	---	P	---
2		10.00	9.995	9.827E+04	---	P	---
3		30.00	28.23	2.617E+05	---	P	---
4		50.00	53.90	4.916E+05	---	P	---
5		75.00	72.57	6.589E+05	---	P	---
6		100.0	100.4	9.082E+05	---	P	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9984
Y = 8.959E+003*X +8.728E+003
X = 1.116E-004*Y -9.743E-001
DL = --- ppb
BEC = 9.743E-01 ppb

Weight: OFF
Min Conc: 0.000

Appendix B7: Calibration curve for Cu



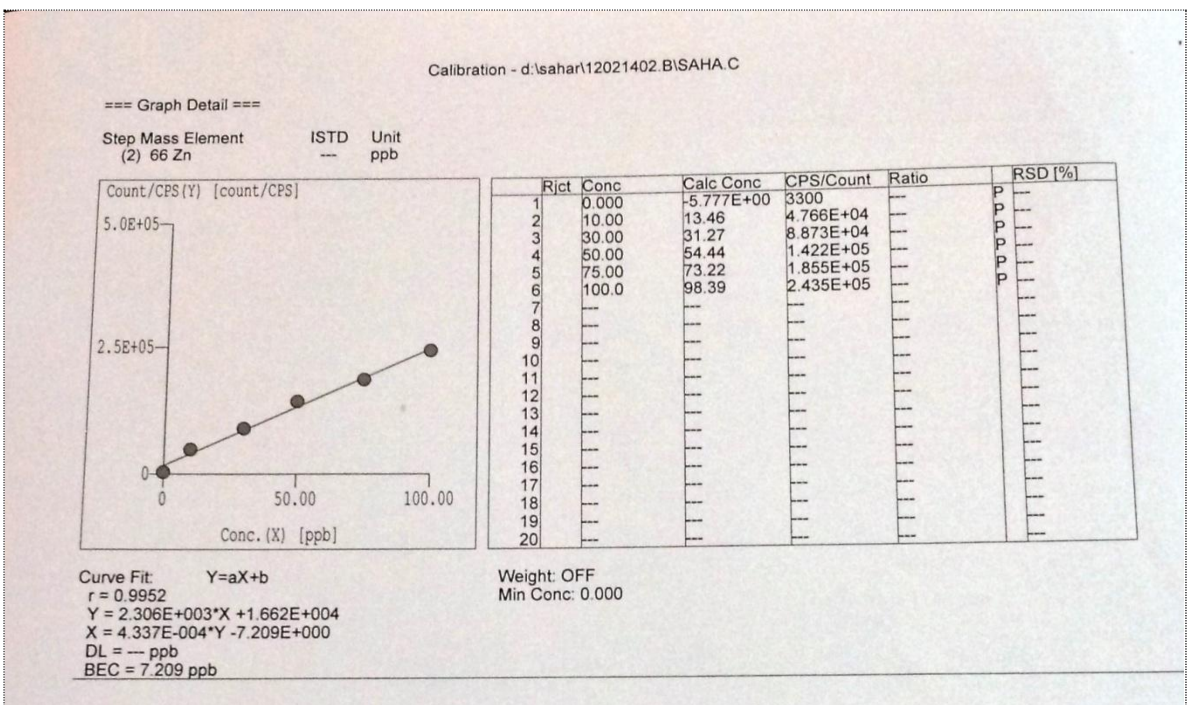
Step Mass Element: (2) 63 Cu   ISTD: ---   Unit: ppb

	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-4.018E+00	2350	---	P	---
2		10.00	13.27	1.690E+05	---	P	---
3		30.00	29.89	3.293E+05	---	P	---
4		50.00	53.07	5.529E+05	---	P	---
5		75.00	73.49	7.498E+05	---	P	---
6		100.0	99.30	9.988E+05	---	P	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9974
Y = 9.644E+003*X +4.110E+004
X = 1.037E-004*Y -4.262E+000
DL = --- ppb
BEC = 4.262 ppb

Weight: OFF
Min Conc: 0.000

Appendix B8: Calibration curve for Zn



Calibration - d:\sahar\12021402.B\SAHA.C

=== Graph Detail ===

Step Mass Element: (2) 66 Zn   ISTD: ---   Unit: ppb

	Rjct	Conc	Calc Conc	CPS/Count	Ratio		RSD [%]
1		0.000	-5.777E+00	3300	---	P	---
2		10.00	13.46	4.766E+04	---	P	---
3		30.00	31.27	8.873E+04	---	P	---
4		50.00	54.44	1.422E+05	---	P	---
5		75.00	73.22	1.855E+05	---	P	---
6		100.0	98.39	2.435E+05	---	P	---
7		---	---	---	---		---
8		---	---	---	---		---
9		---	---	---	---		---
10		---	---	---	---		---
11		---	---	---	---		---
12		---	---	---	---		---
13		---	---	---	---		---
14		---	---	---	---		---
15		---	---	---	---		---
16		---	---	---	---		---
17		---	---	---	---		---
18		---	---	---	---		---
19		---	---	---	---		---
20		---	---	---	---		---

Curve Fit: Y=aX+b
r = 0.9952
Y = 2.306E+003*X +1.662E+004
X = 4.337E-004*Y -7.209E+000
DL = --- ppb
BEC = 7.209 ppb

Weight: OFF
Min Conc: 0.000

Appendix C: Concentration of minerals (µg/g) in sample after calculation

Brand	Type	Na	Mg	K	Ca	Mn	Fe	Cu	Zn
WRA	W	1.287129	62.85446	340.099	3.621782	4.690099	2.415842	0.611881	8.319802
WRA	W	1.326632	60.41302	381.2699	4.91242	5.364252	4.047572	0.795183	9.656648
WRA	W	1.439754	62.87455	391.4982	5.867023	5.210067	3.440349	0.725327	9.933611
WRA	W	1.597815	70.78749	524.5283	4.598808	5.571996	3.223436	0.747766	10.35253
WRA	W	1.343032	67.38222	462.886	3.991489	5.890736	2.514846	0.632423	9.500198
WRA	W	1.398483	70.67578	488.0216	5.583949	5.270513	3.191256	0.848473	9.560791
WRA	W	1.479714	65.2675	460.9189	5.204853	5.348051	2.9286	0.811456	9.291965
WRB	W	1.127219	84.46943	319.1312	5.245562	5.641026	4.230769	0.414201	10.83136
WRB	W	1.17355	64.39436	443.4065	3.513701	6.252979	2.815727	0.52224	10.69599
WRB	W	1.331538	71.48314	330.7391	4.208059	4.389587	4.150209	0.496709	8.837024
WRB	W	1.48892	63.69311	416.8965	3.918678	5.135536	4.084883	0.663831	9.385635
WRB	W	1.672996	66.1743	364.7801	3.618829	4.71717	3.554867	0.634619	8.408955
WRB	W	1.412723	72.81563	392.8912	5.408404	4.370705	2.876497	0.590026	9.918516
WRB	W	1.63848	69.36431	407.9775	4.605054	5.237764	4.11162	0.431755	10.34918
WRC	W	1.265722	137.3777	334.3751	3.375923	3.741266	3.372929	0.784588	9.56678
WRC	W	1.146225	148.2316	370.6925	3.737864	4.312463	3.837924	0.823261	7.331088
WRC	W	1.054134	144.8081	386.3947	6.130906	4.369094	2.768701	0.972441	9.930118
WRC	W	1.603886	153.7718	383.1057	5.492665	4.345757	4.716495	0.878271	12.88065
WRC	W	1.440094	156.9201	372.3952	3.618926	4.663584	1.966358	0.850875	10.86858
WRC	W	1.218269	192.0098	390.043	4.904058	3.85069	3.278033	0.941435	14.74016
WRC	W	1.34646	158.3383	388.7003	3.685362	3.893198	3.485481	0.782617	11.18934
WRD	W	1.21956	98.28648	324.313	3.016234	3.940804	1.994655	0.673134	8.202336
WRD	W	1.734612	136.9694	324.2616	5.108913	4.085731	3.160472	0.805356	8.384293
WRD	W	1.537847	100.738	358.8187	3.974436	3.499101	3.870581	0.83583	9.476733
WRD	W	1.37684	87.53382	313.3914	4.616992	4.13848	4.322523	0.702348	9.519499
WRD	W	1.424436	94.41905	339.0108	5.463166	3.864045	2.406668	0.737672	14.03374
WRD	W	1.56888	84.79584	322.1219	5.171457	3.524281	3.951437	0.767096	11.57384
WRD	W	1.485765	127.6977	351.2465	3.625939	3.961052	3.436141	0.809609	9.874456
WRE	W	1.733728	111.2456	256.0237	4.247535	3.323471	2.052268	0.700197	6.461538
WRE	W	1.998022	119.9832	277.6538	6.283877	3.544016	2.598417	0.776459	11.31652
WRE	W	1.574827	106.0486	280.8801	4.611497	3.507433	2.846383	0.805748	7.354807
WRE	W	1.613288	107.7213	292.6278	6.256216	3.730853	3.396658	0.8703	12.01611
WRE	W	1.64923	115.4787	313.6676	5.454994	3.89163	3.338926	0.854718	7.896763
WRE	W	1.479945	125.8674	288.3896	4.356846	3.605019	2.704011	0.751828	8.468682
WRE	W	1.785714	114.8839	276.9921	6.019841	3.90377	3.343254	0.833333	7.978175
BRA	B	3.448783	949.3828	2143.141	11.7916	9.199372	6.381476	0.858516	18.67053
BRA	B	2.149773	968.9893	2262.684	11.13219	9.526773	9.339063	0.846671	17.27722
BRA	B	5.218078	905.971	2103.107	8.985593	8.471482	5.930531	0.928557	30.45984
BRA	B	4.8867	915.5773	2070.235	7.769458	9.019704	6.031527	1.135961	27.07094
BRA	B	2.879354	944.5099	2162.26	7.263334	11.11691	6.140524	0.989963	24.71069
BRA	B	4.318673	917.8392	2062.919	9.529747	9.138033	6.120165	1.075005	29.05066
BRA	B	3.152303	943.9148	2094.211	9.529388	9.555203	8.355838	0.866759	25.25318
BRB	B	1.773812	843.8296	1152.701	8.708342	10.30566	7.257937	1.281798	20.34313
BRB	B	2.310757	835.9681	1195.288	10.34064	10.21912	8.031873	1.164343	18.34861
BRB	B	3.32967	810.7013	1131.937	13.37862	10.27273	6.193806	1.395604	28.25375
BRB	B	4.275034	881.9713	1190.31	8.236278	10.01476	7.886091	1.066299	16.74405
BRB	B	3.520722	830.7674	1145.22	7.257585	9.985128	6.27206	1.150109	27.73349
BRB	B	3.353586	852.6016	1136.523	6.888446	9.283865	6.473108	0.969124	17.43227
BRB	B	2.518819	850.6458	1125.266	11.59667	9.586965	7.168185	1.251981	18.36173
BRC	B	4.888758	823.1256	882.7486	8.316598	9.299075	7.434534	0.873203	28.75566
BRC	B	3.737463	798.1622	854.6667	8.972468	9.025565	6.989184	0.836775	29.46903
BRC	B	3.204198	816.6251	909.5685	7.485288	9.347783	8.647509	0.913103	18.12475
BRC	B	4.185018	827.793	907.9788	9.316752	9.973457	7.100865	0.83661	24.36689
BRC	B	4.325832	809.8297	886.4728	11.11133	9.163513	7.431786	0.875324	22.51544
BRC	B	4.844745	880.8031	1022.787	7.983678	10.78324	8.4375	0.900677	26.94666
BRC	B	3.854146	844.6863	938.7572	8.666334	10.53447	8.899101	1.066933	29.29271